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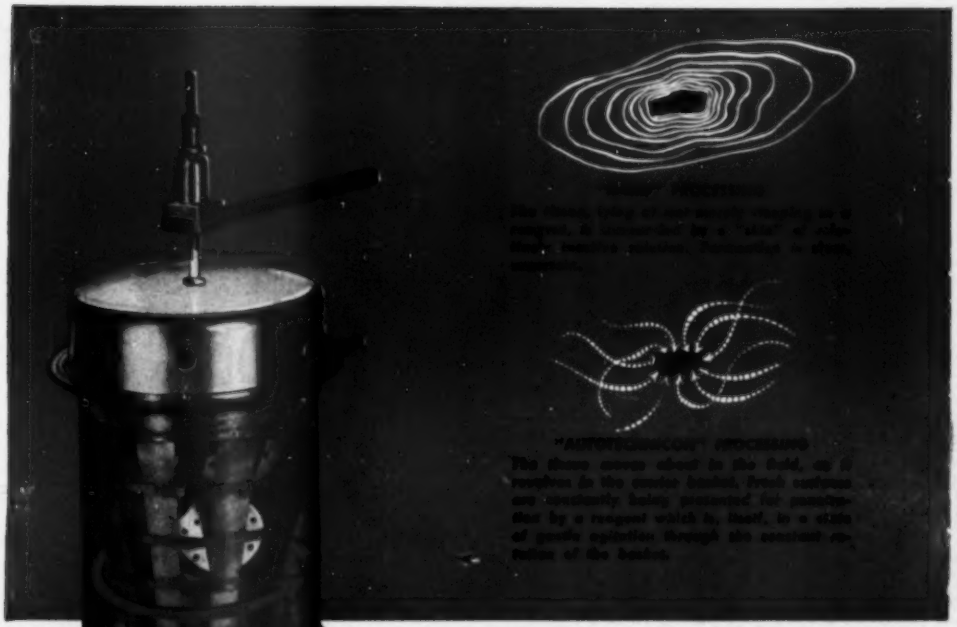

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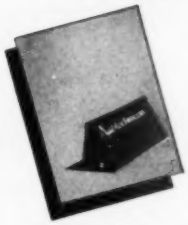

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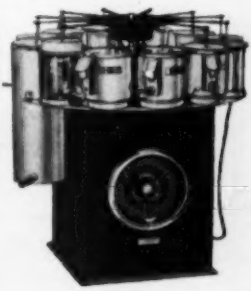

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1. Shapiro, S., Weiner, M., et al.: Am. Heart J. 40:766 (Nov.) 1950

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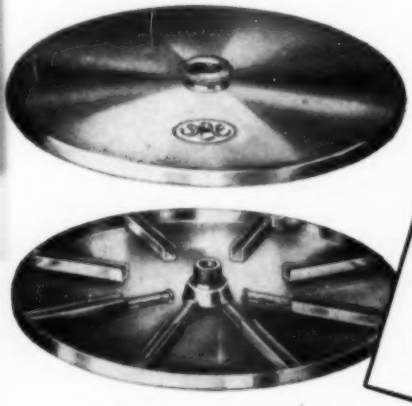

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ARCHIVES OF PATHOLOGY

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NEPHROTOXIC GLOBULIN NEPHRITIS

I. Course After a Single Intravenous Injection

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THE ETIOLOGIC agent of human glomerular nephritis has eluded identification since Bright first associated the occurrence of proteinuria with specific gross lesions of the kidney, in 1827. In recent years, however, this disease has been placed among the hypersensitivity states in consequence of the belief that it is very frequently related to streptococcal infection,[1] a concept which has been widely accepted. Indeed, the Caveltis [2] are said to have produced nephritis in the rat with a mixed antigen of homologous kidney and killed streptococci; Lange and Gold [3] found autoantibodies to human kidney tissue in human patients with glomerular nephritis, and others [4] have found a circulating nephrotoxic substance, presumably a protein, in the serum of human patients with glomerular nephritis.

Since it is desirable to study diseases in the experimental animal, in which conditions may be controlled to a degree not often possible in human subjects, efforts have been made to produce lesions like those of glomerular nephritis in animals by immunologic methods. After the initial studies of Lindemann,[5] Masugi [6] first produced lesions resembling those of nephritis in rats by the administration of antikidney serum.[7] In recent years others have studied the pathogenesis of such nephrotoxic

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Dr. Lippman is a Fellow of the John Simon Guggenheim Foundation.

From the Institute for Medical Research, Cedars of Lebanon Hospital, Los Angeles, and the Gates and Crellin Laboratories of Chemistry (Contribution No. 1617), California Institute of Technology, Pasadena.

This work was aided by grants from the National Heart Institute of the National Institutes of Health, United States Public Health Service, Federal Security Agency, Bethesda, Md., and from Ciba Pharmaceutical Products, Inc., Summit, N. J.

1. Addis, T.: Glomerular Nephritis; Diagnosis and Treatment, New York, The Macmillan Company, 1948, p. 181.

2. Cavelti, P. A., and Cavelti, E. S.: Studies on Pathogenesis of Glomerulonephritis, Arch. Path. **39**:148 (March) 1945; Studies on Pathogenesis of Glomerulonephritis, ibid. **40**:158 (Sept.) 1945.

3. Lange, K.; Gold, M. M. A.; Weiner, D., and Simon, V.: J. Clin. Invest. **28**:50, 1949.

4. Lippman, R. W.; Cameron, G., and Campbell, D. H.: Proc. Nat. Acad. Sc. **36**:576, 1950.

5. Lindemann, W.: Ann. Inst. Pasteur **14**:49, 1900.

6. Masugi, M., and Tomizuka, Y.: Tr. Jap. Path. Soc. **21**:329, 1931.

7. G. W. Wilson and J. Oliver (J. Exper. Med. **32**:183, 1920) produced a similar hemorrhagic glomerulitis in the dog more than 10 years before Masugi's work.

serum nephritis in the rat, notably Smadel and his associates,[8] and Heymann and Lund.[9] Results reported from different laboratories have not been uniform, and this circumstance has been attributed to difference of diet or genetic strain, or unknown variables. However, the investigators used different schedules and routes of administration, slightly different antikidney sera, and allowed different intervals after serum administration for the lesions to develop.

At the start, it should be stated clearly that the lesions produced by administration of nephrotoxic sera are *not* equivalent to those of human glomerular nephritis, and should not be confused with the latter. Although there are many features of correspondence between the two conditions, there are also important differences, such as the failure to find significant hematuria in nephritis due to administration of nephrotoxic serum. Neverless, if constant and reproducible lesions could be secured, it would be possible to study controllable influences on the pathogenesis of this particular experimental nephritis. An analysis of the causes of variability might render the pathogenetic mechanism less obscure. For these reasons, we have investigated the nephritis produced in rats by administration of nephrotoxic globulin and, in this first study, the course after a single, constant intravenous injection will be defined.

METHODS AND MATERIALS

Nephrotoxic Globulin (NTG).—Rabbit anti-rat-kidney serum was prepared by immunizing rabbits for four months with a suspension of rat kidney tissue. Fresh rat kidneys were cut into small pieces with a razor blade, washed with 0.85% sodium chloride solution, and then homogenized in a Waring Blendor.® For each gram of kidney tissue (wet weight), 25 ml. of sodium chloride solution was used. Each rabbit received 0.5 ml. of the whole, freshly agitated suspension twice a week by intraperitoneal injection. The antigen was kept in the frozen state between injections. All manipulations were performed with sterile precautions, and the final antigen suspension contained thimerosal N. F. (merthiolate®) in the proportion of 1:20,000.

After four months of immunization, the rabbits were bled by cardiac puncture. After clotting, the serum was separated by centrifugation, and the antibody globulin was then precipitated by one-third saturation with ammonium sulfate at pH 7.8. The precipitated protein was found by the Tiselius pattern to be composed entirely of gamma globulin. The protein was redissolved, dialyzed against 1.00% sodium chloride solution, and then absorbed at room temperature, first with a half-volume of washed rat erythrocytes, then with a half-volume of washed sheep erythrocytes to remove Forssman antibodies. The final preparation (NTG) was analyzed for protein content by the Kingsley[10] biuret method, and the minimal serological activity was determined by a precipitin test with the soluble portion of the original antigen.

In this experiment, with one exception, only one lot of NTG (3/17/50) was used. The lot contained 13% precipitable antibody and the total protein concentration was 32.5 mg./ml. A single intravenous injection of 0.5 ml. was given to each animal, as described later. In the four-month group, since the stock of NTG (3/17/50) was exhausted, NTG (9/10/50) was used, with 9.2% precipitable antibody and a total protein concentration of 18.5 mg./ml., in a single intravenous injection of 1.2 ml. Since the latter lot was found in other experiments to be more potent than NTG (3/17/50), correction for the error introduced would favor our conclusions.

Control Globulin (GC).—A control globulin (GC) was made, the preparation of which paralleled that of nephrotoxic globulin except that serum from normal, unimmunized rabbits was used as starting material and erythrocyte absorptions were omitted. This preparation had a total protein concentration of 53.0 mg./ml., and an injection of 0.3 ml. was used.

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8. (*a*) Smadel, J. E.: J. Exper. Med. **64**:921, 1936; (*b*) **65**:541, 1937. (*c*) Smadel, J. E., and Farr, L. E.: Ibid. **65**:527, 1937.

9. Heymann, W., and Lund, H. Z.: Science **108**:448, 1948.

10. Kingsley, G. R.: J. Biol. Chem. **131**:197, 1939.

Supernatant Protein Fraction.—After precipitating the serum from immunized rabbits by one-third saturation with ammonium sulfate at pH 7.8 to obtain the nephrotoxic globulin, the supernatant liquid was dialyzed against 1.00% sodium chloride solution, and the volume was adjusted by evaporation in the dialyzing bag at room temperature. This preparation had a total protein concentration of 53.4 mg./ml.

Experimental Procedure.—For these experiments, 143 female rats of the Sherman-Addis strain were used, each weighing 150 gm., with little variation. Groups of six animals were given a single intravenous injection of nephrotoxic globulin, and one group was killed at each of the following intervals: 30 minutes, 2 hours, 4 hours, 6 hours, 24 hours, 1 week, 4 weeks, and 4 months. Except at the first two intervals, a four-hour urine collection was made just prior to each kill, and during the collection period the animals received only 10% dextrose solution with 0.4% sodium chloride and 0.5% betaplexin.[9] [10a] Otherwise, all the animals were fed our stock diet, which contains 17% protein, and received water ad libitum. The four-month group was weighed daily but the other groups were weighed only at the beginning and the end of the experiments. An identical procedure was followed with groups of animals that received control globulin in an equivalent dose of total protein. In addition, identical groups received 0.5 ml. of 0.85% sodium chloride solution (SC) by intravenous injection, except for the omission in this category of the four-month group.

Two groups received a single intravenous injection of the supernatant protein fraction. One group received 0.3 ml., with the total protein given comparable to that given the animals that received nephrotoxic and control globulin. The other group received 1.0 ml. A urine collection was made for four hours after the injection, and the animals were then kept for four weeks.

At the end of each experiment a four-hour urine collection was made, and the animals were killed by exsanguination from the abdominal aorta, during light ether anesthesia. The following data were obtained: wet weight of kidneys, heart, and adrenal glands. Clotted blood and an oxalated sample for hematocrit (Wintrobe) determination were obtained. The four-hour urine volume was measured, and the serum was examined for lipemia which, when present, was graded from 1 to 4+.

The urine was centrifuged for five minutes at 1,650 rpm, and the sediment was examined microscopically. The supernatant urine was pooled in each group for a determination of protein excretion by the biuret method of Kingsley.[10] The total serum protein concentration was determined by the copper sulfate-specific gravity method.[11] The creatinine concentration was determined in serum and in urine by the method of Bonsnes and Taussky,[12] and from these values the endogenous creatinine clearance was calculated.

Tissues of the kidney and adrenal glands were fixed for microscopic examination in 10% formalin in 0.85% sodium chloride solution. Paraffin sections were stained with hematoxylin and eosin and by the Mallory technique, to demonstrate connective tissue elements. Sections from typical blocks were also stained by the periodic acid-Schiff technique. Frozen sections of the kidneys and adrenals were stained for fat with Sudan III.

RESULTS AND COMMENT

General Observations.—Immediately after the injections of nephrotoxic globulin (NTG), control globulin (GC), or 0.85% sodium chloride solution (SC), the animals recovered from anesthesia promptly, in less than one minute. Those which had received sodium chloride solution or control globulin behaved normally, but those which had received nephrotoxic globulin were obviously ill, with drooping posture, increased irritability, closed eyes, and cold extremities. This condition was still present at six hours, but at 24 hours they seemed normal, and they behaved normally from that time on. None of the animals had edema or ascites.

10a. This is a preparation containing factors of the B complex, made by Winthrop-Stearns, Inc., New York.

11. Phillips, R. A.; Van Slyke, D. D.; Dole, V. P.; Hamilton, P. D., and Archibald, R. M.: Copper Sulfate Method for Measuring Specific Gravity of Whole Blood and Plasma, New York, Josiah Macy, Jr., Foundation, 1945.

12. Bonsnes, R. W., and Taussky, H. H.: J. Biol. Chem. **158**:581, 1945.

There was a short period of oliguria following the injections of sodium chloride solution and control globulin, after which the animals voided urine that was normal in appearence. By contrast, after the injections of nephrotoxic globulin the animals were all totally anuric for at least two hours. The initial voiding then appeared normal, but the second and succeeding voidings had a deep-red or red-brown color in more than one-half of the cases. The color was obviously due to hemoglobin, and this judgment was confirmed by a strongly positive benzidine reaction. At the end of 24 hours the urine had become free of hemoglobin, but the yellow color was deeper than normal as a result of bilirubinuria. Later observations revealed no abnormal coloration of the urine.

At first it was thought that intravascular hemolysis had been caused by antibodies formed against the small amounts of blood present in kidney tissue used as an antigen. Blood specimens obtained from animals with hemoglobinuria at early intervals showed increasing hemoglobinemia, with bilirubinemia as the hemoglobinemia waned. Portions of nephrotoxic globulin have been treated by repeated absorptions with rat and sheep erythrocytes, under various conditions, and so far it has proved impossible to separate the hemolytic factor from the nephrotoxic factor. Some previous investigators—for instance, Smadel [8] and Pressman and Keighley [13]—have observed "hematuria" after the administration of nephrotoxic serum. However, in at least one instance the investigator [14] acknowledged the possible use of an erroneous word, since he had probably observed hemoglobinuria. Others, such as Solomon and associates [15] (who perfused the kidneys used for antigen preparation in order to wash out the blood), have not observed hematuria and have not mentioned the occurrence of hemoglobinuria.[16] These observations are of some interest in view of the report of Evans and associates [17] that erythrocytes of patients with glomerular nephritis, like those of patients with acquired hemolytic anemia, are sensitized to "anti-globulin."

Body Growth.—The animals that were given nephrotoxic globulin did not gain weight as rapidly as those that were given control globulin. This can be seen in Figure 1, which demonstrates the daily weights over a four-month period. The nephritic animals maintained a steady growth lag of a little more than 10%. Although one is tempted to attribute the lag to urinary loss of protein that is necessary for growth, relative growth remained constant while the relative proteinuria changed during the four-month period, as will be noted. An alternative explanation is that the total protein-synthesizing capacity of the body is taxed to replace the urinary protein loss and cannot maintain the necessary rate of synthesis for normal growth. These animals were not uremic or azotemic and, although food consumption was not measured, appetite and food consumption appeared grossly normal.

13. Pressman, D., and Keighley, G.: J. Immunol. **59**:141, 1948.

14. Pressman, D.: Personal communication to the authors.

15. Solomon, D. H.; Gardella, J. W.; Fanger, H.; Dethier, F. M., and Ferrebee, J. W.: J. Exper. Med. **90**:267, 1949.

16. In our experience, perfused organs, though thought to be "blood free" on gross examination, still contain many erythrocytes and leucocytes that may be stained and seen in tissue sections on microscopic examination. For this reason we chose to remove antibodies to blood elements by absorption.

17. Evans, R. S.; Takahashi, K.; Duane, R. T.; Payne, R., and Liu, C-K.: Primary Thrombocytic Purpura and Acquired Hemolytic Anemia: Evidence for a Common Etiology, A. M. A. Arch. Int. Med. **87**:48 (Jan.) 1951.

Organ Weight.—In all the experiments a slight rise in the adrenal gland weight was noted during the first 24 hours after injection, with return to the previous value at one week, and another slight rise at four months. However, there were no significant differences between the animals which had received sodium chloride solution, control globulin, or nephrotoxic globulin.

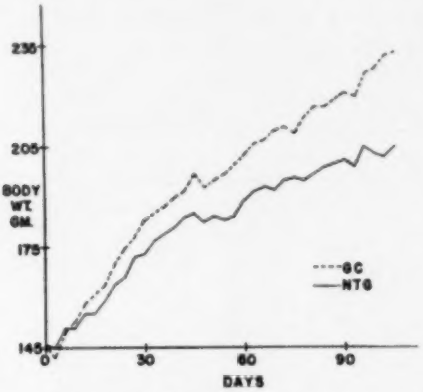

Fig. 1.—Growth of rats after the administration of nephrotoxic globulin *(NTG)* or control globulin *(GC)* in a single intravenous injection.

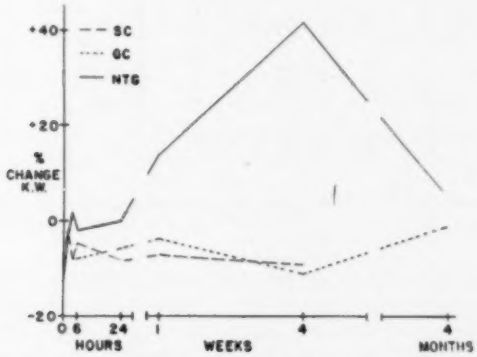

Fig. 2.—Change in kidney weight (*K. W.*) of rats after the administration of nephrotoxic globulin *(NTG)*, control globulin *(GC)*, or sodium chloride solution *(SC)* in a single intravenous injection, compared with the kidney weight predicted for a normal rat of the same size. (Addis, T., and Gray, H.: Growth **14**:49-81, 1950).

In the animals which received nephrotoxic globulin a moot increase in kidney weight occurred within the first few hours, a manifest increase was observed at one week, and a great increase was present at four weeks. At the end of four months the kidneys had nearly returned to normal size relative to body weight (Fig. 2). No significant changes occurred in kidney weight after administration of sodium chloride solution or control globulin.

After administration of nephrotoxic globulin there was also an increase in heart weight at one week and at four weeks, but the heart weight had returned to the control value at four months (Table 1). This observation suggests the possibility that administration of nephrotoxic globulin produced a transitory hypertension, which diminished at the end of the period observed. Rather [18] has shown that cardiac hypertrophy may occur as early as two days after unilateral nephrectomy with ligature constriction of the remaining kidney and is directly proportional to the degree of hypertension observed.

Proteinuria.—Neither the animals given sodium chloride solution nor those given control globulin had significant proteinuria during the experiments. After injection of nephrotoxic globulin there was a slight increase in protein excretion

TABLE 1.—*Body and Organ Weights After a Single Intravenous Dose of Nephrotoxic Globulin*

Interval	Final Body Wt., Gm.	Kidney Wt., Mg.	% Change from Predicted* Kidney Wt.	Adrenal Wt., Mg.	% Change from Predicted Adrenal Wt.	Heart Wt., Mg.	% Change from Predicted Heart Wt.
Sodium Chloride Solution Controls							
30 min.	155	1,019	— 6	44	— 4	558	— 1
2 hr.	149	1,021	— 3	41	— 11	566	+ 3
4 hr.	151	980	— 8	44	— 4	583	+ 7
6 hr.	144	980	— 5	50	+ 11	526	0
24 hr.	150	1,011	— 8	43	— 8	558	— 1
1 wk.	165	1,047	— 7	44	— 10	618	+ 6
4 wk.	190	1,111	— 9	49	— 2	660	+ 2
Globulin Controls							
30 min.	154	956	— 11	37	— 21	559	+ 1
2 hr.	149	1,035	— 2	40	— 13	533	— 1
4 hr.	154	995	— 8	45	— 4	586	+ 6
6 hr.	149	978	— 9	43	— 7	550	+ 2
24 hr.	157	1,023	— 6	42	— 13	551	— 2
1 wk.	161	1,066	— 4	46	— 4	594	+ 4
4 wk.	182	1,059	— 11	48	— 4	626	0
4 mo.	231	1,356	— 1	58	+ 16	710	— 5
Nephrotoxic Globulin							
30 min.	156	946	— 13	42	— 12	560	0
2 hr.	147	1,012	— 4	47	+ 2	566	+ 6
4 hr.	147	1,077	+ 2	47	+ 2	540	+ 1
6 hr.	143	1,018	— 2	47	+ 6	544	+ 4
24 hr.	147	1,044	0	46	0	554	+ 4
1 wk.	156	1,246	+ 14	45	— 6	628	+ 12
4 wk.	177	1,094	+ 42	52	+ 4	680	+ 13
4 mo.	222	1,423	+ 6	55	+ 10	702	+ 5

* For purposes of comparison, the observed organ weights were compared with the organ weight predicted for a normal rat of this colony under stock conditions (Addis, T., and Gray, H.: Growth **14**: 49 and 81, 1950).

even in the first voided urine (determined in those animals which did not have hemoglobinuria). The proteinuria rapidly increased in the first 24-hour interval but reached maximal values after one week (Fig. 3). After four weeks the proteinuria had subsided and at four months, although still substantial, the proteinuria was less than half of the peak value.

Total Serum Protein Concentration.—In the animals given sodium chloride solution and control globulin there were no significant changes in the total serum protein concentration except for a rise at the end of four months. The latter observation can be explained by the rise of total serum protein concentration that occurs with normal growth in the rat.[19] In the animals given nephrotoxic globulin there

18. Rather, L. J.: Am. J. Physiol. **159**:153, 1949.

19. Lippman, R. W.: Proc. Soc. Exper. Biol. and Med. **67**:193, 1948.

was a fall in the total serum protein concentration at the same time that the proteinuria reached its height. As the proteinuria declined with time, the total serum protein concentration rose and at the end of four months had become higher than the initial value but was still lower than the four-month value for the animals that received control globulin (Table 2).

Serum Creatinine Concentration and Endogenous Creatinine Clearance.—No significant or consistent changes were noted in the serum creatinine concentration or in the endogenous creatinine clearance when the latter was calculated in terms of body weight. However, when calculated in terms of the kidney weight found at autopsy the endogenous creatinine clearance had declined to a minimum at four weeks and had nearly returned to control values at the end of four months. From this information it would appear that the renal enlargement, to be described, resulted from an increase in nonfunctional renal mass (with respect to creatinine excretion), with a diminished excretion capacity per unit of renal weight, although the organ

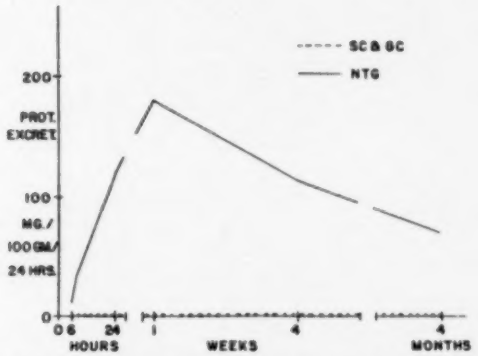

Fig. 3.—Protein excretion of rats after the administration of nephrotoxic globulin (*NTG*), control globulin (*GC*), or sodium chloride solution (*SC*) in a single intravenous injection.

in toto remained capable of meeting the excretory load, as indicated by failure of the serum creatinine concentration to rise.

Urine Volume.—No significant changes were noted except for the transient anuria that followed injection of nephrotoxic globulin, which lasted for about two hours and was followed by oliguria for about two hours. After injections of sodium chloride solution and control globulin only a short period of oliguria ensued.

Hematocrit Values.—Because of the complicating factor of hemolysis, no significance could be attached to hematocrit values, and these are not reported.

Urinary Sediment.—The results of examinations of the sediment in the urine of rats are difficult to evaluate, for several reasons. Rat urine is normally more alkaline than human urine, so that casts may dissolve and erythrocytes may undergo lysis. It is difficult to collect urine uncontaminated with hair, dander, feces, and food particles, which further confuse the examination. In addition, just as the rat normally excretes more protein in relation to body weight, so it excretes more casts and erythrocytes than a normal human subject. In the case of the animals which had received nephrotoxic globulin, the sediment contained hyaline, granular, and

fatty casts, blood casts, epithelial cells (some containing fat), and a few erythrocytes. None of these elements was present in large numbers. While the animals that had been given sodium chloride solution and control globulin seemed to have a still more sparse sediment, nevertheless all the elements mentioned could also be found in many of the specimens. No certain quantitative difference in the sediment could be measured.

Gross Anatomy of the Organs.—In the animals which had received sodium chloride solution and control globulin, no abnormalities were noted in any of the organs, with the exception of hydronephrosis. This condition was observed with about the same frequency (10%) and the same characteristics (usually unilateral occurrence, and without evidence of bacterial infection or urinary obstruction) as in the normal Slonaker-Addis animals.

TABLE 2.—*Serum Protein Concentration and Renal Functions After a Single Intravenous Dose of Nephrotoxic Globulin*

Interval	Urinary Protein Excretion, Mg./100 Gm. Body Wt./24 Hr.	Total Serum Protein, %	Serum Creatinine Concentration, Mg./100 Cc.	Endogen. Creatinine Clearance, Ml./100 Gm. Body Wt./Min.	Endogen. Creatinine Clearance, Ml./Gm. Kidney Wt./Min.
Sodium Chloride Solution Controls					
30 min.	..	5.76	0.80	.....	.....
2 hr.	..	5.25	0.85	.....	.....
4 hr.	1	5.25	0.65	0.448	0.690
6 hr.	1	5.25	0.59	0.338	0.494
24 hr.	0	5.25	0.64	0.398	0.622
1 wk.	1	5.25	0.51	0.311	0.490
4 wk.	0	5.40	0.78	0.273	0.467
Globulin Controls					
30 min.	..	5.76	0.78	.....	.....
2 hr.	..	5.40	0.56	.....	.....
4 hr.	3	5.25	0.60	0.495	0.766
6 hr.	1	5.25	0.59	0.425	0.648
24 hr.	0	5.30	0.64	0.396	0.561
1 wk.	0	5.25	0.55	0.413	0.624
4 wk.	1	5.75	0.87	0.244	0.420
4 mo.	3	6.95	0.56	0.434	0.741
Nephrotoxic Globulin					
30 min.	..	5.40	0.89	.....	.....
2 hr.	..	5.04	0.64	.....	.....
4 hr.	12	5.04	0.74	0.283	0.386
6 hr.	34	5.04	0.57	0.404	0.567
24 hr.	121	4.68	0.82	0.257	0.362
1 wk.	183	4.75	0.55	0.384	0.500
4 wk.	115	5.12	0.92	0.244	0.259
4 mo.	71	5.81	0.69	0.392	0.610

No gross changes were observed in the kidneys until six hours after the administration of nephrotoxic globulin. At that time the kidneys were somewhat pale, and two of them showed dark surface mottling. At 24 hours the kidneys again appeared normal, but at one week they were obviously enlarged and pale. The surface was smooth, and the capsule stripped easily. Sagittal section revealed a very pale cortex, but the medullary striations appeared normal. At four weeks the kidneys were grossly enlarged and very pale, with a granular surface. The cortex was very pale and narrow with respect to the total sagittal section area. A few petechiae were seen on the surface and in the cortical substance. At four months the kidneys were normal except for a mottled appearance of the surface. However, there was no granularity, and the capsules stripped with only slight difficulty.

Microscopic Anatomy of the Kidneys and Adrenal Glands.—No significant differences between groups were seen in the adrenal glands, which were stained with hematoxylin and eosin, Mallory's connective tissue stain, and Sudan III. The lipoid material stained with Sudan III, although variable within each group, had approximately the same range of variability whether the animals had received sodium chloride solution, control globulin, or nephrotoxic globulin.

In general, at the early time intervals no distinction could be made between the kidneys of animals which had received sodium chloride solution and those of animals which had received control globulin. Glomeruli, tubules, blood vessels, and interstitial tissue appeared normal (Fig. 4*A*). This was also true concerning the animals which had received sodium chloride solution, at the end of one week, four weeks, and four months. However, at these times, in the animals which had received control globulin the glomeruli were slightly more cellular and contained less blood, a granular eosinophilic precipitate was present in the subcapsular spaces, and a moderate degree of tubular degeneration was present (Fig. 4*B*).

The kidneys of animals which had received nephrotoxic globulin could be distinguished at all times. Even 30 minutes after the administration of this globulin the glomeruli seemed relatively bloodless, with increased cellularity. There was a moderate amount of lightly eosinophilic granular material in the subcapsular spaces. The proximal convoluted tubules showed slight intraluminal desquamation of cytoplasm. At the end of two hours the sections were similar to the 30-minute sections, but within or on some of the capillary loops in the glomeruli there was a homogeneous, deeply eosinophilic material (fibrinoid). At four hours and six hours the glomeruli were less cellular and contained more blood than the earlier specimens and the tubules showed little abnormality. At 24 hours the glomeruli were relatively bloodless and much more cellular than normal, and the nuclei in both the epithelial and the endothelial cells varied in size, shape, and staining qualities. Some of the nuclei were larger than normal and densely hyperchromatic, while some had a very irregular shape. There was a granular precipitate in the subcapsular spaces and the deeply eosinophilic material, first observed at the end of two hours within or on the glomerular capillary loops, had become much more pronounced (Fig. 5*A*). The tubules showed a considerable amount of intraluminal desquamation, and the lumina were larger than normal. No significant changes were observed in the blood vessels or in the interstitial tissue. No deposits of lipoid material were noted.

At one week after administration of nephrotoxic globulin the picture closely resembled that found at the end of 24 hours. At the end of four weeks the glomeruli were bloodless and more cellular than normal, and had thickened basement membranes. Eosinophilic material was precipitated in the subcapsular spaces, and the previously mentioned deeply eosinophilic coagulum within or on the glomerular loops, which resembled fibrinoid, was present in considerable amount. In some glomeruli a crescent of this fibrinoid material lined a portion of the capsule, and in others a crescent capped the glomerulus in one portion or another. There was advanced epithelial degeneration, with desquamation of the cytoplasm. The proximal tubular epithelium was low-cuboidal or flat, and the tubules had relatively large lumina (Fig. 5*B*). In the collecting tubules there was a variable amount of eosinophilic homogeneous material. With the Mallory stain, the changes noted were

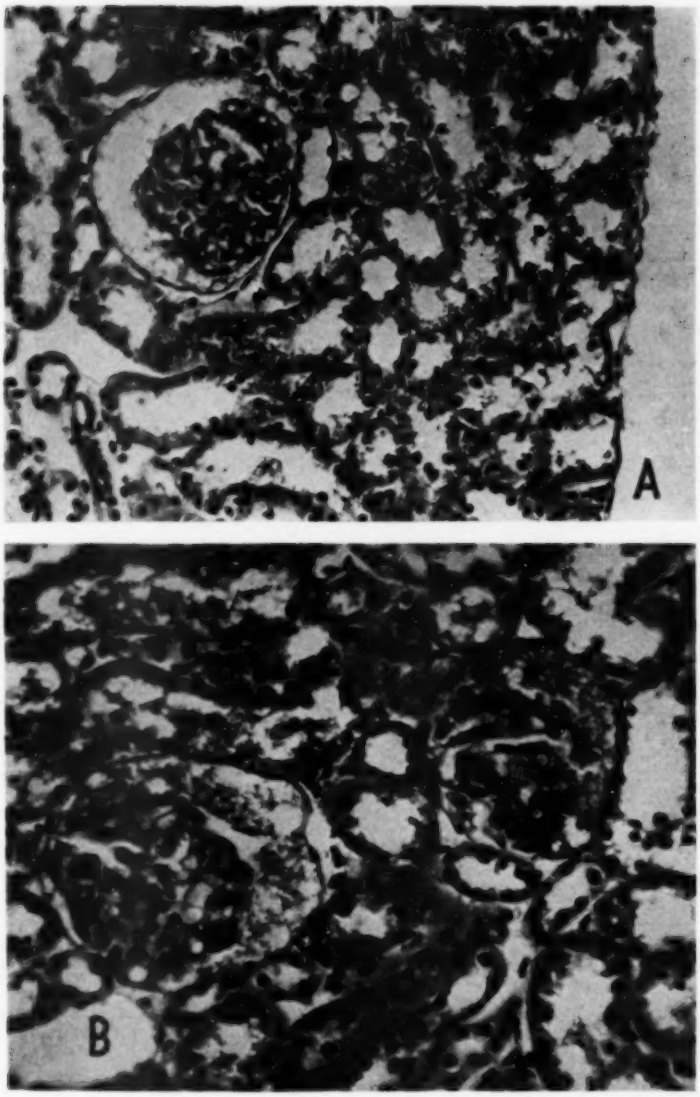

Fig. 4.—*A*, section of rat kidney one week after a single intravenous injection of sodium chloride solution. Hematoxylin and eosin stain; × 240.

B, section of rat kidney one week after a single intravenous injection of control globulin. An eosinophilic precipitate is seen in the subcapsular space, and some tubular degeneration is evident. Hematoxylin and eosin stain; × 240.

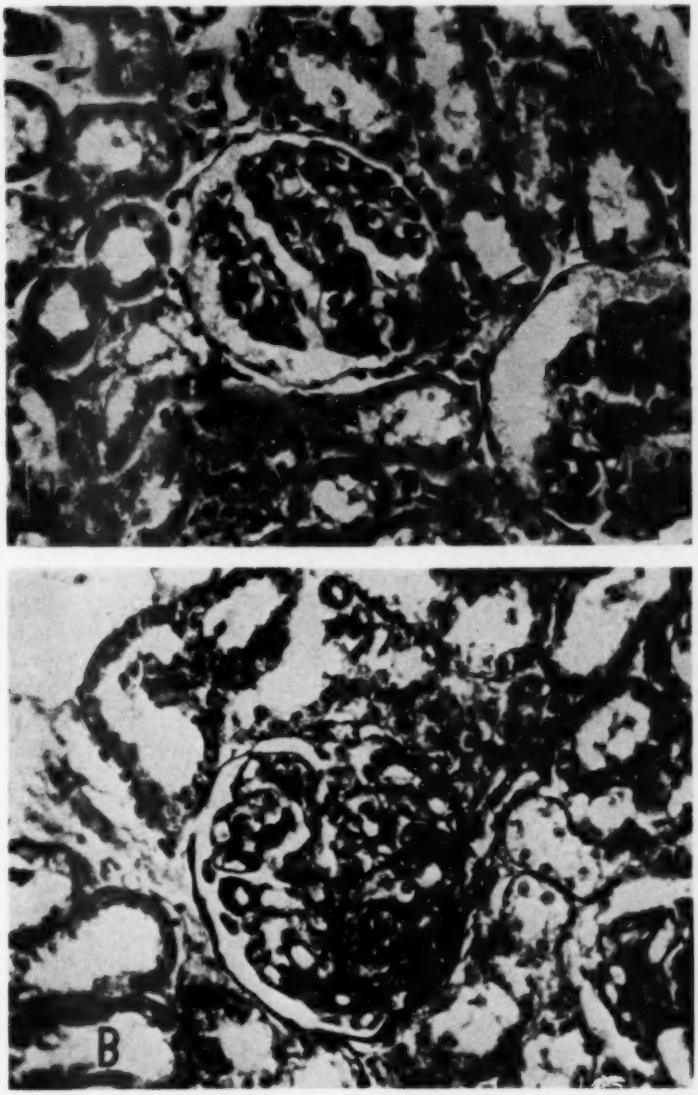

Fig. 5.—*A*, section of rat kidney one week after a single intravenous injection of nephrotoxic globulin. The glomeruli show increased cellularity with variable nuclei. At the arrow is a deposit of fibrinoid material. Hematoxylin and eosin stain; × 240.

B, section of rat kidney four weeks after a single intravenous injection of nephrotoxic globulin. The glomerular basement membrane is thickened, and on the right is a deposit of fibrinoid that appears nearly black. The tubules show low epithelium with large lumina. Periodic acid-Schiff stain, aniline blue-orange G counterstain; × 240.

more readily seen and the material which resembled fibrinoid stained a deep red, but no significant change was seen in the interstitial connective tissue or in the blood vessels. Very little additional deposit of lipoid material was seen in the Sudan III preparations. Occasional small deposits were seen in tubular lining cells and in the glomerular tufts as well, but these deposits were infrequent, requiring a search for their demonstration. There was a striking disproportion between the gross enlargement of some kidneys and the relatively mild lesions seen at microscopic examination. It seemed obvious that the increased renal size could not be attributed to fat deposition, inflammation, or fibrosis. Representative sections that were stained by the periodic acid-Schiff technique demonstrated nothing more than has been already described.

At four months after administration of nephrotoxic globulin a qualitative change from the condition at four weeks had occurred. This was readily seen on gross inspection of sections stained by the Mallory technique, which at four weeks were predominantly red-brown but at four months were predominantly blue. In the sections stained with hematoxylin and eosin, eosinophilic granular precipitate was seen in the subcapsular spaces, which were smaller than normal. The glomeruli showed increased cellularity and the capillaries of the tuft were often bloodless. Occasional polymorphonuclear leucocytes could be seen between the capillaries. The capsular basement membrane appeared to be thickened. The epithelium and the endothelium of both membrane and capillaries were swollen. Occasional glomerular adhesions and epithelial crescents capping the glomerular tufts were seen. The tubules showed pronounced degeneration, though not as severe as that seen in the sections at one week and four weeks. The proximal tubular epithelium had ragged cytoplasm with pyknosis and karyolysis. The lining epithelium was low-cuboidal in most places, with some desquamation in lumina that appeared larger than the normal. Occasionally, pink-staining homogeneous material could be seen in the lumina. There were also evidences of regeneration, with variation in nuclear size, some unusually large, hyperchromatic nuclei, and some mitoses. The Mallory stain showed the thickened basement membrane well, both in the glomerular capsule and in the tubules. There was a moderate amount of collagen formed in the glomerular tufts and in the medullary interstitial tissue, and this was in contrast to the sections from earlier intervals, in which collagen was present in normal amounts. In addition, the deeply eosinophilic "fibrinoid" material, which was so prominent in the one-week and four-week sections, was completely absent. Sections stained for fat were similar to those at the earlier intervals, though the fat deposits were slightly more frequently observed.

Supernatant Protein Fraction.—The animals which received the supernatant protein fraction, even those which received 53.4 mg. total protein, did not show any significant proteinuria following the injection or at the end of four weeks. Microscopic sections of the kidneys were indistinguishable from the kidney sections of animals which had received injections of sodium chloride solution.

Since the supernatant protein fraction had no nephrotoxic activity so far as this could be determined, it may be concluded that the nephrotoxic antibody produced by immunizing rabbits with rat kidney tissue resides wholly within the gamma globulin fraction of the serum proteins.

CONCLUSIONS AND COMMENT

It is obvious from published descriptions and photographs that the functional and structural lesions described here are generally similar to those produced by Smadel,[8b] Heymann,[9] and others. Differences may be attributed to various factors. Smadel used immature, prepubescent rats and did not distinguish between the sexes. The amount of nephrotoxic serum administered was variable, and the route of administration was not specified. The animals were killed at irregular intervals. Heymann, likewise, did not specify the sex of his animals, and used immature, prepubescent animals. Variable amounts of nephrotoxic serum from several different lots were used, and the animals were killed at irregular intervals. Preliminary evidence, which will be reported in a later paper, indicates that important differences depend on sex, amount of nephrotoxic serum, and route of administration. Heymann noted that the disease varied with "individual susceptibility of the rats." This variability has been observed by others and related to the level of adrenal function.[20] From their data, which are derived from animals that are often not strictly comparable with one another, Smadel and Heymann concluded that the disease produced in their experiments was progressive in some animals. From the physiologic and pathologic data presented in this study the conclusion may be reached that the nephritis produced after a single intravenous injection of nephrotoxic globulin is continuing but not progressive.

The damage may be observed histologically in a very short time, as reported by Heymann and Lund.[21] In 30 minutes tubular desquamation has begun, the glomerular space contains proteinaceous material, and capillary ischemia may be seen in the glomerular tufts. These observations are not remarkable when it is remembered that in the mouse the circulating plasma is cleared of nephrotoxic antibody 18 minutes after intravenous injection.[22]

The possibility must be noted that such a single insult might produce progressive disease if the period of observation were extended. One possible cause of progression is the continuing proteinuria. Notwithstanding the work of Baxter and Cotzias,[23] there is some reason to believe that long-standing, severe proteinuria may in itself cause progressive renal damage.[24] In addition, temporary periods of nutritional disturbance may also cause progressive renal disease,[25] and the animals that receive nephrotoxic globulin certainly endure some gross nutritional disturbance, possibly as a consequence of the massive proteinuria (Fig. 1). However, in this experiment the morphologic damage reached a peak in one week and then receded, with evidence of repair in the form of collagen deposits and restoration of the tubular epithelium. The proteinuria also receded, and the creatinine excretion returned to normal. This is a reasonable course to expect after the administration of a single insult with an exogenous noxious material. Damage is done almost instantly, and this produces a continuing disease, but the changes, after the initial injury and response, are recessive, not progressive.

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The reason for renal enlargement under the conditions of this experiment is not entirely clear. As previously mentioned, the enlargement did not seem to be explained adequately by deposition of fat, inflammatory exudate, fibrosis, or deposition of carbohydrate-containing substances. Further study is necessary to determine whether the increase in renal weight can be attributed to an increase in water content or to true growth, with an increase in the total kidney protein content.

Recent work has been advanced to show that the significant antigen in kidney tissue is derived from the glomeruli [26] and that the antibody formed is principally bound by the glomeruli.[27] This view does not exclude the possibility of tubular damage following the administration of nephrotoxic serum. The peculiar circulatory anatomy of the kidney requires that the preponderance of blood which perfuses the tubules must first pass through the glomerulus. Consequently, damage to the glomerulus with associated vascular spasm could theoretically produce tubular ischemia and, if sufficiently prolonged, necrosis. The appearance of sections from the 30-minute and two-hour groups suggests that such ischemia of the glomeruli and tubules may actually occur. However, the work mentioned above does not exclude the possibility of direct tubular damage from antitubule antibodies or from other antibodies to renal antigens. In Pressman's work, for example, since the nephrotoxic antibodies perfuse the glomeruli first, with a low concentration of antibody it is possible that none reaches the tubules until glomerular combining sites are saturated, or that rapid vascular spasm may prevent antibody from reaching the tubules at early time intervals. Other work has shown that the nephrotoxic globulin exerts a direct toxic effect on tubular cells in tissue culture explants, in which secondary circulatory effects are excluded.[4]

SUMMARY

A detailed description is given of the course taken by functional and structural renal lesions produced in the rat by a single intravenous injection of nephrotoxic globulin.

The nephrotoxic activity of rabbit anti-rat-kidney serum resides wholly in the gamma globulin fraction.

The lesions produced, mild proliferative glomerulitis with moderate tubular degeneration, are continuing but not progressive. The damage reaches a peak rapidly, with the first signs as early as 30 minutes after administration of nephrotoxic globulin and with maximum evidence of damage at one week. After that the lesions recede, with some evidence of repair.

Dr. Harry Goldblatt examined and interpreted tissue sections for this study. Prof. Jean Oliver contributed helpful criticism. E. Elmo Jacobs, Ruth Taniguchi, and Jay Banovitz gave technical assistance.

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26. Greenspon, S. A., and Krakower, C. A.: Direct Evidence for Antigenicity of Glomeruli in Production of Nephrotoxic Serums, Arch. Path. **49**:291 (March) 1950. Krakower, C. A., and Greenspon, S. A.: Localization of the Nephrotoxic Antigen Within the Isolated Renal Glomerulus, ibid. **51**:629 (June) 1951. Solomon and others.[18]

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PATHOLOGICAL CHANGES IN ACUTE AND IN PROTRACTED VITAMIN A DEFICIENCY

Their Prevention by the "Lard Factor"

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SIGNS of vitamin A deficiency develop very rapidly (within one month) in rats when the vitamin A-low diet fed contains rancid lard as the source of fat.[1] This effect of rancid lard is prevented by the supplement of liberal amounts of known forms of vitamin A or by the "lard factor."[2a] No ill effects of feeding of rancid lard are demonstrable if the increased requirements of vitamin A are satisfied.

The present report deals with histopathological changes which result from the rapid depletion of vitamin A. A parallel study was made in rats fed a diet moderately low in vitamin A without added rancid lard. On the latter regimen the nutritional disease appeared only after 3 to 10 months. Both the acute and the prolonged vitamin A deprivation led to the development of the customary epithelial lesions. In addition, renal tubular changes were found in acutely deficient animals and adrenal cortical alterations were observed in rats partially deprived of vitamin A for a long period. The development of all of these changes could be prevented by the oral feeding of vitamin A palmitate or cod liver oil or of the "lard factor."

METHODS AND MATERIALS

Albino rats of the Sherman strain from a highly inbred colony were used. For the "acute" experiments, the mother rats were transferred within two days of the birth of a litter from "Rockland rat diet" to the diet given in Table 1. The lard had been made rancid by heating to 100 C. and aeration. Its peroxide number was 256. Freshly rendered lard without added preservatives was molecularly distilled at 215 C. and 10^{-3} mm. Hg.[3] Seven per cent was taken

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From the Merck Institute for Therapeutic Research, Rahway, N. J., and the Departments of Pathology and Animal Care, College of Physicians and Surgeons, Columbia University, New York.

1. Kaunitz, H., and Slanetz, C. A.: Relation of Vitamin A and "Lard Factor" to Disease Caused by Rancid Lard, Proc. Soc. Exper. Biol. & Med. **75**:322, 1950.

2. Kaunitz, H., and Slanetz, C. A.: (*a*) An Unknown Factor with Vitamin A Activity Distilled from Lard, J. Nutrition **42**:375, 1950; (*b*) ibid., Table 2, p. 377.

3. This distillate was prepared and supplied by Distillation Products Industries, Rochester, N. Y.

off as the "distillate" containing the more volatile fractions; the rest formed the "residue." The vitamin-A-like activity of a freshly rendered lard ("lard factor") can be concentrated in the distillate fraction.

In the "chronic" experiments, a diet was prepared containing 30% lactalbumin and 10% residue fraction as fat source; 5,000 units of crystalline beta-carotene [4] per kilogram was added. This diet was originally meant to serve as a control ration. However, it was found that the animals became vitamin A-deficient after 3 to 10 months on the diet. This was due to the fact that, although the ration was kept refrigerated, only traces of carotene were chemically found several days after the preparation of the diets. This was probably a consequence of the removal of the biological antioxidants from the residue fraction by the distillation and the omission of free tocopherols in the preparation of the diets. Careful studies of the distillate by any of the conventional methods for known forms of vitamin A revealed it to contain no measurable amounts of these substances.

In order to avoid the possible destructive effect of rancid lard, a suspension of all known factors of the B complex [2b] and ascorbic acid was fed to the rats by dropper twice weekly, in addition to the supplements in the diet.

TABLE 1.—*Rancid Lard Diet*

Basal Mixture	
"Cerelose" (dextrin)	54%
Alcohol-extracted casein	30%
"Cellu-flour" (roughage)	2%
Salt mixture	4%
Rancid lard	10%
Lard residue or lard distillate	5%
Supplements *	**Mg./Kg.**
Inositol	1,000
Choline	1,000
p-Aminobenzoic acid	300
Nicotinic acid	100
Calcium pantothenate	10
Thiamine hydrochloride	2
Riboflavin	4
Pyridoxine	4
Alpha-tocopherol acetate	25
Free alpha-tocopherol	30
Calciferol	10γ

* Dr. Leo A. Pick, of Hoffman-LaRoche, Inc., Nutley, N. J., supplied us with most of the synthetic vitamins used, including the synthetic vitamin A palmitate. The calciferol was supplied by Dr. M. L. Tainter, of the Sterling-Winthrop Research Institute.

The young were weaned at 21 to 24 days; paired littermates of comparable weights were used to make up groups. Autopsies were performed at the dates indicated below after the animals had been killed by intraperitoneal injections of pentobarbital sodium. Slices of various organs were immediately fixed in Bouin's solution, except for the central nervous system, which was placed *in toto* in 10% formalin (40% formaldehyde solution).

OBSERVATIONS

In the "acute" experiments, in which rancid lard was fed, the animals grew at a rate 30 to 60% below that of the controls for the first three weeks; thereafter they lost weight and died after 30 to 50 days on the diet. Paralysis of the animals (Table 2) was frequently observed in this group; however, in later groups its incidence varied greatly. When the residue in the diet was replaced by 5% distillate or when either 1,000 units weekly of synthetic vitamin A palmitate or liberal amounts of cod liver oil were fed, normal growth and reproduction were observed.

4. Crystalline beta-carotene was supplied by Barnett Laboratories, Long Beach, Calif.

When the amount of distillate was reduced to 1 to 2%, various reproductive failures were noted, ranging in severity from inability to become pregnant, to resorptions, to the birth of a dead litter.

In the "chronic" experiments, the majority of the rats died after being restricted to the diet for three to six months. Some of them survived for about one year. Growth was considerably slowed, the deficient group weighing, after three months, approximately 100 gm. when the weight of the animals protected with distillate was 150 gm. About one-third of the chronically deficient animals eventually lost weight before they were killed.

Epithelial lesions typical of vitamin A deprivation [5] were present in most rats of both groups. Their incidence in the two experimental groups is listed in Table 2. They were present in variable intensity and often involved several of the tissues of the same animal, in which case the lesions were counted only as incidence. Listed in order of frequency, the metaplastic changes were observed in the following sites: cornea, renal pelvis, urinary bladder, trachea, endometrium, and bronchus. In two

TABLE 2.—*Observations on Vitamin A-Deficient Rats*

Group	Days on Diet	Rats	Symptoms*		Pathological Findings*			
			Wt. Loss	Paralysis	Squamous Cell Metaplasia	Secondary Infection	Renal Tubular Changes	Hyaline Droplets in Adrenal
Acute								
A	30	4	...	...	...	...	...	...
B	31-35	10	6/10	5/10	5/9	3/9	3/9	...
C	40-47	7	7/7	6/7	7/7	2/7	1/4	...
Chronic								
A	107-117	7	2/7	...	4/7	3/7	...	3/7
B	134-144	5	2/5	...	2/5	2/5	...	1/3
C	160-171	5	2/5	...	3/5	3/5	...	3/5
D	226-329	2	2/2	...	0/2	1/2	...	2/2

* The denominators of all fractions indicate the total number of animals examined, the numerator, the frequency of the observed symptom.

instances metaplastic changes of the pancreatic ducts were observed in the chronic experiment. In many instances, the epithelial lesions were associated with infections of the organs involved in the metaplastic changes. Metaplasia of the corneal epithelium almost invariably led to keratitis, iritis, and even complete destruction of the eye. Tracheitis, pyelitis, cystitis, or endometritis was found in association with squamous cell metaplasia of the respective epithelium, so that in many instances the infection was obviously related to the inadequate protection afforded by defective lining, although occasionally metaplasia could be seen without infection and inflammatory lesions without evidence of metaplasia. In paralyzed animals of the first group there was evidence of discrepant growth of the central nervous system and its bony capsule as described by Wolbach and Bessey.[6] The pressure effect appeared to be mutual, and degenerative changes of nerve tissue occurred, together with pressure atrophy of vertebral or cranial bone.

5. For brevity, a discussion of the literature is omitted. Most of the relevant literature can be found in a work by Richard H. Follis Jr. (The Pathology of Nutritional Diseases, Springfield, Ill., Charles C Thomas, Publisher, 1948).

6. Wolbach, S. B., and Bessey, O. A.: Tissue Changes in Vitamin Deficiencies, Physiol. Rev. **22**:233, 1942.

In the second group, in which the deficiency developed slowly over a prolonged period, no paralysis was observed and there were no changes in the central nervous system except in one animal which showed a purulent infection of the middle ear extending to the temporal lobe.

In the present experiments, two histological changes were found which to our knowledge have not been observed in vitamin A deficiency. A peculiar renal tubular lesion (Fig. 1) was present in about one-third of the acutely deficient rats but not in the chronically deficient group. "Hyaline droplet" changes of adrenal cortical cells (Fig. 2) were seen in one-half of the animals with protracted deficiency and

Fig. 1.—Desquamation of renal tubular epithelium. High-power view of kidney of a rat rendered acutely deficient in vitamin A.

apparently were strictly dependent on the prolonged course of the nutritional disease. Since the tubular changes also occurred in animals on a diet containing "residue" instead of rancid lard, the alterations do not appear to be due to a toxic effect of rancid lard.

In rats with the acute deficiency the loops of Henle frequently showed enlargement of the lining cells with conspicuous clear nuclei and basophilic cytoplasm. Desquamation of these cells appeared evident, and conglomerates, often lamellated, of well-preserved tubular epithelium could be seen in the lumen of some of the tubules. Distal convoluted and collecting tubules were markedly distended and

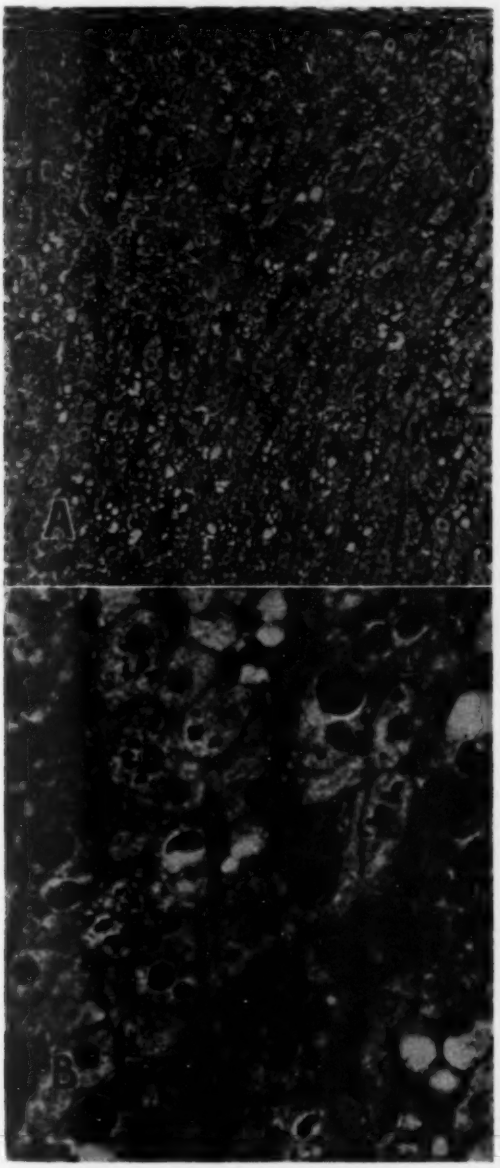

Fig. 2.—"Hyaline droplet" changes in adrenal cortical cells: *A*, low-power view of adrenal cortex of a rat chronically deficient in vitamin A. *B*, high-power view of same section (periodic acid-Schiff stain).

contained conglomerates of ill-defined cellular debris often in a concretion-like formation. The lining cells of such tubules were not of the usual type but much larger, with conspicuous hyperchromatic nuclei, and more numerous, as if by excessive regeneration. Not infrequently they exhibited multinucleated epithelial giant cells, such as are seen in man in association with Bence Jones proteinuria. Whether these tubular changes represent another type of manifestation of epithelial metaplasia or are related to some injurious action of an unknown agent is uncertain. It is unlikely that the tubular changes are a consequence of ascending infection, since in most instances they were found in the absence of pyelitis; neither were they seen in the "chronic" animals, many of which showed pyelitis and pyelonephritis.

In many of the rats of the second group the cytoplasm of the cells of the adrenal cortex contained eosinophilic droplets of somewhat increased refractility, which varied greatly in size and number. These cytoplasmic inclusions gave a positive staining reaction with the periodic acid-Schiff reagent and with metachromatic stains like toluidine blue or thionin. Variegated color reactions were obtained with Mallory's azocarmine, some of the droplets staining red, others yellow or orange, and still others blue. Some of the cortical cells contained numerous small droplets, while in others, possibly by confluence, a single large droplet replaced almost the

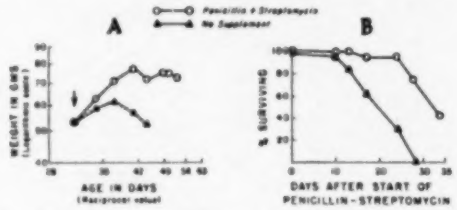

Fig. 3.—Effect of penicillin and streptomycin on growth and survival of rats rendered acutely deficient in vitamin A.

entire cell. In cases where this change was extreme, whole groups of cortical cells had disappeared and aggregations of a homogeneous material giving stain reactions identical with the droplets were found in their place. It is likely that this material, after destruction of the cortical cells, was freed and accumulated in the spaces vacated by the cells. When the "lard factor" at the above-noted level or vitamin A palmitate or cod liver oil was fed, none of the above changes could be observed.

The high rate of infections in various organs secondary to the metaplastic epithelial changes demonstrates that the consequences of infection must play an important part in the "clinical" picture of the disease; they may be of importance for the not fully understood cause of death in vitamin A deficiency. This possibility was tested with an attempt to counteract the infections by penicillin-streptomycin treatment.

Eight groups of well-matched littermates were restricted, on weaning, to the rancid lard diet (Table 1) minus the residue supplement. Half of the animals received daily intraperitoneal injections of 1.8 mg. of crystalline penicillin and 3 mg. of streptomycin in 0.2 cc. saline solution; the controls, only the saline. Their growth and survival rate were observed. The growth curves of two corresponding groups are given in Figure 3 *A*. The differences were statistically significant. In the

remaining groups, the differences always went in the same direction but were not always statistically significant. The survival rates of these groups (Fig. 3*B*) were combined. In the individual groups, the supplemented animals always survived their controls significantly. A total of 25 supplemented and 26 unsupplemented animals were used.

In view of the improvement of growth and survival rate brought about in these vitamin A-deprived rats by penicillin-streptomycin treatment, the possibility that secondary infection is one of the main causes of the death of vitamin A-deficient rats seems strongly supported.

SUMMARY

The use of rancid lard as a source of fat in vitamin A-deficient diets leads to rapid development of the characteristic "clinical" and histopathological signs of vitamin A deficiency. All changes can be prevented by the administration of synthetic vitamin A palmitate or a molecular distillate of lard containing the "lard factor" which is chemically different from the known forms of vitamin A.

In many of the "acutely" deficient animals, renal tubular changes hitherto not described were noted which perhaps represent another metaplastic change. In chronically deficient animals, the appearance of hyaline droplets in the adrenal glands was observed in about half of the animals.

The metaplastic changes were frequently accompanied with secondary infections which were thought to influence the "clinical" course of the fatal disease. The protective effect of penicillin-streptomycin treatment of the deficient animals gave support to this idea.

INDUCTION OF THYROID CANCER IN THE RAT BY RADIOACTIVE IODINE

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AND
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IN EARLIER investigations[1] we observed suspicious cytological changes in the thyroid parenchyma of rats killed at various intervals after they had received single injections of I^{131}. In order to evaluate the significance of these changes, a study of the late effects of ionizing radiations emitted by this isotope was undertaken. We had already noted the presence of thyroid neoplasms in two rats that were killed 18 months after a single injection of 400 μc of I^{131}. Although these growths resembled thyroid cancers in man, the question of their being cancerous was left open, because criteria other than the histological appearance of the tumors were lacking.[2] Conclusive evidence that radioactive iodine can induce thyroid cancer in the rat is presented here. Thyroid cancer was encountered in 7 of a group of 25 rats that had received single I^{131} injections. In five of the seven so affected, metastatic lesions were found in one or more of the following tissues: lung, bone, adrenal gland, lymph node, and subcutaneous tissue. Evidence is also presented to show that thyroid cancer in the strain of rat studied is not produced by prolonged thyrotropic hormone stimulation. The conclusion is drawn that the ionizing radiations emitted by I^{131} are carcinogenic in the rat.

EXPERIMENTAL PROCEDURE

Male Long-Evans rats that had been raised on stock diet No. 1,[3] containing approximately 1 γ of iodide per gram, were used throughout.

I^{131} Experiment.—At the age of 3 months 25 rats received a single intraperitoneal injection of 400 μc of I^{131} and were killed 1.5 to 2 years later. They were anesthetized with pentobarbital sodium and exsanguinated. A block of cervical tissue ventral to the prevertebral fascia and caudal to the upper margin of the larynx was removed and sectioned serially at 10 μ. Representative portions of the various organs were also prepared for histological examination.

From the Division of Physiology, University of California School of Medicine.

1. Goldberg, R. C.; Chaikoff, I. L.; Lindsay, S., and Feller, D. D.: Histopathological Changes Induced in the Normal Thyroid and Other Tissues of the Rat by Internal Radiation with Various Doses of Radioactive Iodine, Endocrinology **46**:72, 1950. Goldberg, R. C., and Chaikoff, I. L.: Cytological Changes That Occur in the Anterior Pituitary Glands of Rats Injected with Various Doses of I^{131} and Their Significance in the Estimation of the Thyroid Function, ibid. **46**:91, 1950.

2. Goldberg, R. C., and Chaikoff, I. L.: Development of Thyroid Neoplasms in the Rat Following a Single Injection of Radioactive Iodine, Proc. Soc. Exper. Biol. & Med. **76**:563, 1951.

3. Taurog, A., and Chaikoff, I. L.: The Relation of the Thyroxine Content of the Thyroid Gland and of the Level of Protein-Bound Iodine of Plasma to Iodine Intake, J. Biol. Chem. **165**:217, 1946.

Hematoxylin-eosin and Masson's trichrome stain were used after formalin fixation. Osseous tissue was decalcified in 0.5% formic acid. Wilder's method was employed to demonstrate reticulum.

In selected animals, 10 μc of carrier-free I^{131} was injected intraperitoneally 24 hours before the rats were killed. Radioautographs were prepared by floating a 4 μ section onto an Eastman medium lantern slide and exposing it for two to four days. These sections were stained with iron-hematoxylin and metanil yellow.

Propylthiouracil Experiment.—Six-week-old rats were placed on the stock diet,[3] to which had been added 0.2% propylthiouracil. These rats were killed at intervals from 3 to 32 months thereafter. Their thyroids and representative sections of liver, kidney, and lung were prepared for histological examination.

RESULTS

I^{131} Experiment

Thyroid tumors were found in 9 of the 25 rats that were killed from 1.5 to 2 years after they had received a single intraperitoneal injection of 400 μc of I^{131}. The tumors in seven of these rats were considered carcinomas as judged by histo-

TABLE 1.—*Distribution of Thyroid Neoplasms in I^{131}-Treated Rats*

	Malignant Tumors				Benign Tumors		
Rat	Alveolar Adeno-carcinoma	Papillary Adeno-carcinoma	Small Cell Carcinoma	Spindle Cell Carcinoma	Fetal Adenoma	Follicular Adenoma	Metastases
1	1	..	1	..	..	1	None
2	..	..	1	..	..	..	Lung, skin
3	..	..	1	..	..	..	Lung, sternum, adrenal gland
4	1	..	..	..	..	..	None
5	1	..	..	..	..	2	Lung
6	..	1	..	..	..	1	Lymph nodes
7	..	..	..	1	..	..	Lung
8	..	..	..	..	..	3	None
9	..	..	..	..	1	2	None

logical appearance and by the presence of metastases. Benign thyroid adenomata were found in five rats, three of which also had malignant tumors.

These tumors have been classified according to their resemblance to human thyroid neoplasms. Four types of malignant tumors were found: (1) alveolar adenocarcinoma; (2) papillary adenocarcinoma; (3) small cell carcinoma, and (4) spindle cell carcinoma. Two types of benign tumors were encountered: (1) fetal adenoma and (2) follicular adenoma. The distribution of these tumors among the rats is shown in Table 1.

Malignant Tumors.—Alveolar Adenocarcinoma: This tumor, which occurred in three rats (1, 4, and 5, Table 1), was composed of closely packed, large, pale, polyhedral, cells that were only occasionally arranged in acini, which were devoid of colloid (Fig. 1*A*). The nongranular cytoplasm was somewhat basophilic. The nuclei were pale-staining, chromatin-poor, and exhibited considerable pleomorphism; mitotic activity was low. The tumors were only partially encapsulated. Vessels occluded with tumor tissue were noted. In Rat 5, a metastasis was found in the lungs.

Papillary Adenocarcinoma: This tumor, found only in Rat 6, closely resembled the papillary adenocarcinoma seen in man. The eosinophilic columnar cells con-

tained hyperchromatic nuclei, which showed little pleomorphism. Little or no colloid was observed in the follicles. Dark, round, calcific concretions (psammoma bodies) were of frequent occurrence in the highly vascular stroma; some were also found in the alveoli. Metastases were found in the cervical lymph nodes. The primary tumor and the metastases failed to concentrate injected radioiodine.

Small Cell Carcinoma: This highly anaplastic neoplasm (Fig. 1*B* and *C*) was found in three rats (1, 2, and 3). It was composed of tightly packed small cells, arranged only rarely in an acinar fashion. The nuclei were hyperchromatic and

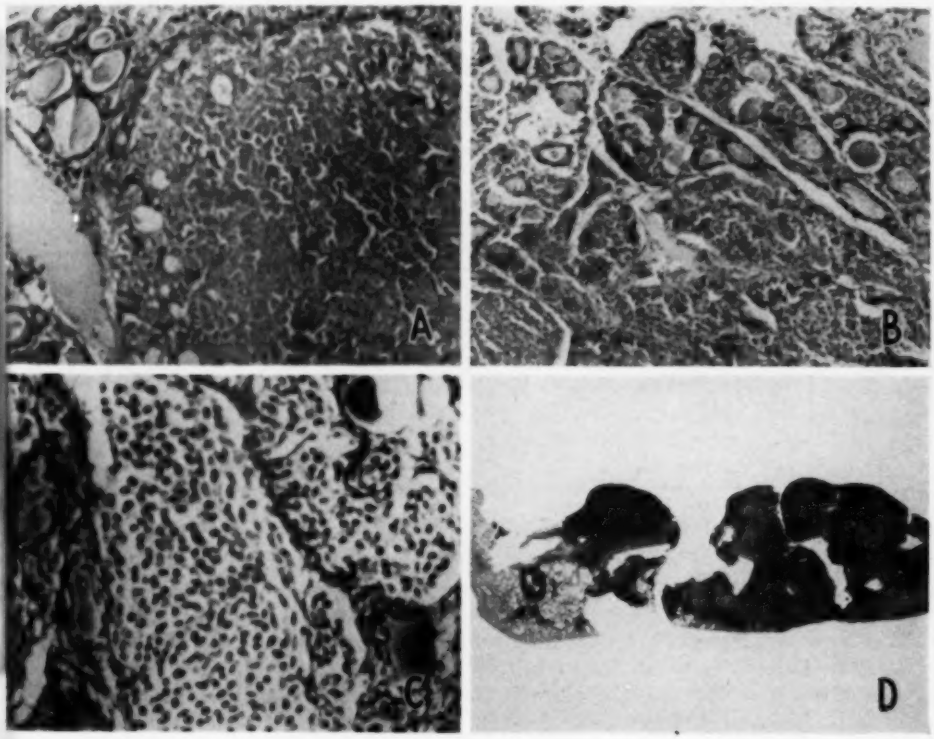

Fig. 1.—*A*, portion of the alveolar adenocarcinoma from Rat 5. This tumor had metastasized to the lungs from the thyroid. Noncolloid-containing acini are seen at the periphery of the tumor. Hematoxylin and eosin; × 100.

B, small cell carcinoma found in the thyroid of Rat 2. Note the highly undifferentiated, invasive character of this neoplasm. Hematoxylin and eosin; × 100.

C, higher magnification of portion of tumor shown in *B*. The reticulum distribution is seen to be very sparse. Wilder's stain; × 200.

D, pulmonary metastasis of a small cell carcinoma of the thyroid (Rat 2). A portion of normal lung tissue is seen at the left. Hematoxylin and eosin; × 4.

pleomorphic, and showed high mitotic activity. Numerous atypical mitotic figures were also noted. The tumor was not encapsulated and was invading the adjacent tissue; vascular invasion was noted in all three rats.

Metastases of the thyroid carcinoma were found in Rats 2 and 3. In Rat 2 one metastasis was found in the lung (Figs. 1*D* and 2*A*) and another metastasis, weighing 71 gm.s was found in the subcutaneous tissues (Fig. 2*B* and *C*). Acini were far more common in the metastases than in the primary tumor. In Rat 3, metastases were noted in the lungs, the sternum, and the adrenal gland (Fig. 2*D*).

Radioautographs of sections from the pulmonary and subcutaneous metastases revealed blackening above background only in the few acini that had redifferentiated.

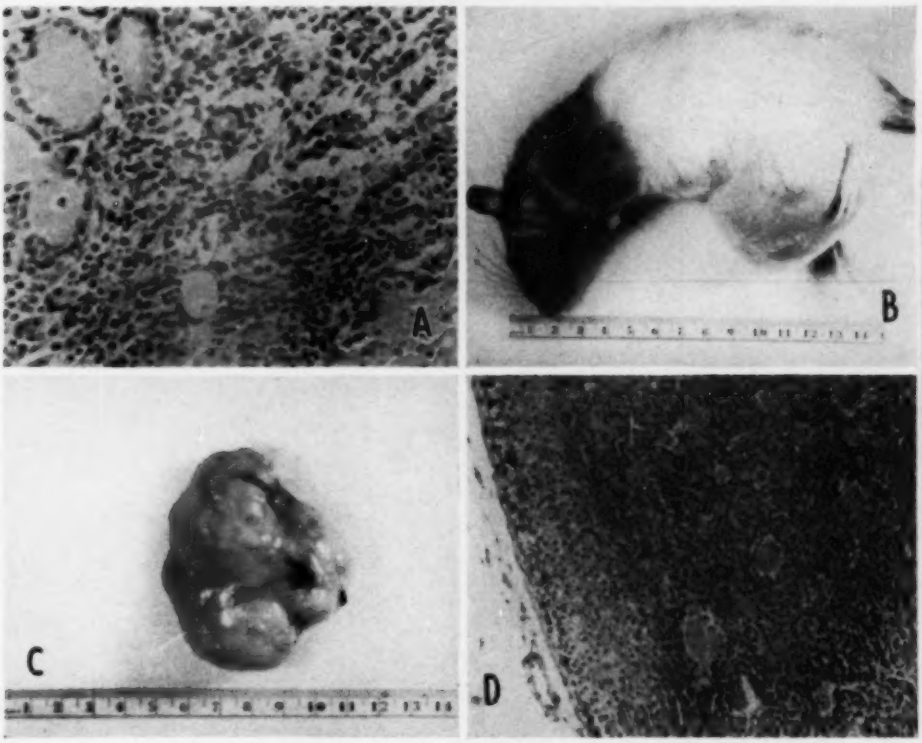

Fig. 2.—*A*, higher magnification of the tumor metastasis shown in Figure 1*D*. Note the colloid-containing follicles which had redifferentiated. These follicles were the only areas to show blackening on radioautographs. Hematoxylin and eosin; × 200.

B, Rat 2, which had a small cell carcinoma of the thyroid and metastases in the lungs and subcutaneous tissue.

C, metastasis found in subcutaneous tissue in Rat 2.

D, adrenal metastasis of small cell carcinoma of thyroid found in Rat 3. Only a rim of cortical tissue remains. The occasional follicles showed blackening on the radioautographs. Hematoxylin and eosin; × 100.

Spindle Cell Carcinoma: Although some areas of the primary thyroid tumor found in Rat 7 resembled the small cell type described above, the major portion of it resembled the spindle cell type seen in man and was therefore classified as a

separate type. The reticulum distribution was sparse, and acinous formation was quite rare. The tumor gave no evidence of iodine-concentrating capacity. A large metastasis was found in the lungs.

Benign Tumors.—Two major types of adenomata, fetal and follicular, were found in five rats (Table 1 and Fig. 3*A*). These resembled, histologically, those described in the rat by several previous investigators.[4]

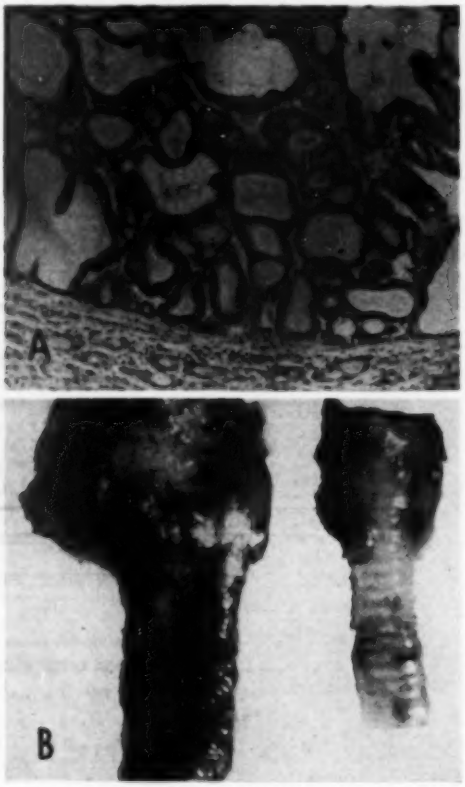

Fig. 3.—*A*, thyroid adenoma from Rat 8. This benign, nonmetastasizing tumor is also found in goitrogen-treated rats and in very old normal rats. A thin rim of nontumorous thyroid tissue is seen at the lower border of the adenoma. Hematoxylin and eosin; × 100.

B, left, tremendously hypertrophied thyroid taken from a rat maintained on propylthiouracil for 32 months. This gland weighed 1,500 mg. and had markedly compressed the adjacent cervical tissues, bulging up through the infrahyoid muscles and extending down into the mediastinum. Right, thyroids from a control normal rat. They weighed 34 mg.

4. Griesbach, W. E.; Kennedy, T. H., and Purves, H. D.: Studies on Experimental Goitre: VI. Thyroid Adenomata in Rats on Brassica Seed Diet, Brit. J. Exper. Path. **26**:18, 1945. Money, W. L., and Rawson, R. W.: The Experimental Production of Thyroid Tumors in the Male Rat, Tr. Am. A. Study Goitre, 1947, p. 171. Lacquer, G. L.: Nodular Hyperplasia of Thyroid Glands Induced by Thiouracil, Cancer Res. **9**:247, 1949.

Propylthiouracil Experiment

Since part of the thyroid gland had been destroyed by the injected I^{131}, we raised the question whether the thyroid cancers observed here were the result of thyroid stimulation by increased amounts of thyrotropic hormone released by the anterior pituitary gland. To study this question, we fed rats the propylthiouracil-containing diet described above.

One hundred twenty-five such rats were killed between 18 and 32 months after the start of the goitrogen feeding. The thyroid glands of these rats were tremendously enlarged (Fig. 3*B*), and in many animals the glands bulged between the infrahyoid musculature, extended down into the thorax, and effected marked compression of all adjacent structures, including the trachea. The gland weights showed a progressive increase with time (Table 2); indeed, glands from animals killed at the end of the study (32 months) were as much as 50 times as large as those of the controls.

Table 2.—*Weights of Thyroid Glands of Rats Maintained on Propylthiouracil for from Twelve to Thirty-Two Months*

Rats	Period on Propylthiouracil, Months	Body Weight, Gm.	Thyroid Gland Weight, Mg.
60	12-14	140-174	194-339
60	14-18	136-168	184-650
40	18-24	130-170	210-635
40	24-30	120-158	243-790
25	26-32	134-165	309-1510
20	None *	350-450	30-42

* These rats were maintained on the stock diet,[3] to which no propylthiouracil had been added, and were killed at the age of 33.5 months.

Microscopic examination of the thyroids of these goitrogen-treated rats revealed the typical picture of epithelial hypertrophy and hyperplasia, loss of colloid, increased vascularity, and hypertrophy of the small arteries and arterioles.

In 24 of the 125 animals, atypical thyroid parenchymal growths were found. These were discrete, well demarcated nodules, generally deep within the gland and encapsulated by thin, fibrous connective tissues. These tumors (adenomata) presented a benign histological appearance, resembling closely the benign tumors noted under the heading "Benign Tumors." Metastases were not found in any of these tumor-bearing, goitrous rats.

In none of the goitrous animals were neoplastic lesions of the thyroid other than adenomata found.

Comment

As pointed out earlier,[2] the incidence of cancer of the thyroid gland even in old rats is extremely low. The frequency of thyroid carcinomata in our I^{131}-treated rats therefore shows that the growths could not have arisen spontaneously.

Evidence that thyrotropic hormone is not the causative agent is provided by the results of the study with propylthiouracil. Although the thyroids of the rats were subjected to continuous stimulation by high levels of endogenous thyrotropic hormone for periods of well over 2.5 years, thyroid cancer was not found in a single animal. That a continuous high titer of thyrotropic hormone stimulated the thyroids

of these animals is clearly shown by the tremendous hypertrophy and hyperplasia of these glands and by the stimulated condition of the epithelium. It is interesting to note that the rat pituitary can continue to synthesize high levels of thyrotropic hormone for periods up to 2.5 years, and that the thyroid gland apparently has an unlimited capacity for hypertrophy and hyperplasia.

In the rats that had cancerous thyroids, a considerable amount of apparently viable thyroid tissue still remained. Much of this tissue was atypical only in that many of the cells contained yellow pigment granules; the epithelium did not appear to be unusually active as judged by cell height, vascularity, and colloid content. These findings also suggest that an unduly high level of thyrotropic hormone was not present in these rats.

It is a reasonable inference that the agent responsible for induction of the thyroid cancers is the ionizing radiation of the I^{131} trapped by the gland. That neoplasia can be induced by radiation is well established. This subject has recently been reviewed by Brues.[5] It is of interest in this connection that Koletsky, Bonte, and Friedell [6] found a high incidence of malignant neoplasms in rats long after they had received a single large dose of P^{32}.

Doniach [7] reported that radioactive iodine increases the incidence of thyroid adenomata in normal and goitrogen-treated rats. He also found evidence of cancer in one of the thyroid neoplasms observed in a group of five goitrogen-treated rats that had received injections of 32 μc of I^{131}.

SUMMARY

In 7 of 25 rats that had received single intraperitoneal injections of 400 μc of I^{131} carcinoma of the thyroid gland developed 1.5 to 2 years later.

Metastases were found in five of the seven rats with thyroid carcinoma. The areas invaded by the metastases were lung, lymph node, bone, adrenal gland, and subcutaneous tissue.

5. Brues, A. M.: Carcinogenic Effects of Radiation, Recent Adv. Biol. Med. Physics **2**:171, 1951.

6. Koletsky, S.; Bonte, F. J., and Friedell, H. C.: Production of Malignant Tumors in Rats with Radioactive Phosphorus, Cancer Res. **10**:129, 1950.

7. Doniach, I.: Effect of Radioactive Iodine Alone and in Combination with Methylthiouracil and Acetylaminofluorene upon Tumor Production in the Rat's Thyroid Gland, Brit. J. Cancer **4**:223, 1950.

COMPARISON OF AGING PROCESSES IN THE RENAL AND SPLENIC ARTERIES IN THE NEGRO AND WHITE RACES

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ST. LOUIS

IN PREVIOUS reports,[1] the patterns of aging changes in several major human arteries, particularly those changes dealing with elastic tissue breakdown, proliferation, and mineralization, were described and compared. It was pointed out that the location of lipid plaques might be related to the focal severity of these elastic tissue changes, and it was suggested that lipids might even be released locally as a product of chemical processes associated with the changes in elastic tissue and calcium. Other studies dealing with the chemistry of the aging of elastic fibers derived from the aorta tend to substantiate this concept.[2]

Comparative studies of aging processes in various components of the vascular wall, especially the internal elastic lamella, seem to be particularly important, since investigations dealing with lipid-metabolic factors in the genesis of arteriosclerosis fail to explain a predilection of atheroma formation for specific areas of the aorta and the coronary and cerebral arteries, as well as a considerably lower frequency of lipid plaque formation in the pulmonary, hepatic, splenic, and renal arteries. Furthermore, such experiments have failed to produce degenerative changes resembling those occurring in the so-called muscular arteries of man. In general, the peculiarities of the distribution of atheroma formation point out the importance of localizing factors which have heretofore received relatively little consideration.

Furthermore, the association observed between increasing severity of arteriosclerosis and advancing age in humans represents a parallelism which is not adequately explained in the experiments in which atherosclerosis has been produced by

From the Snodgras Laboratory, St. Louis City Hospital; Department of Pathology, Homer G. Phillips Hospital; Department of Pathology, Jewish Hospital, and Department of Pathology, St. Louis University School of Medicine.

1. (*a*) Blumenthal, H. T.; Lansing, A. I., and Wheeler, P. A.: Am. J. Path. **20**:665, 1944. (*b*) Lansing, A. I.; Blumenthal, H. T., and Gray, S. H.: J. Gerontol. **3**:87, 1948. (*c*) Blumenthal, H. T.; Lansing, A. I., and Gray, S. H.: Am. J. Path. **26**:989, 1950. (*d*) Blache, J. O., and Handler, F. P.: Coronary Artery Disease: A Comparison of the Rates and Patterns of Development of Coronary Arteriosclerosis in the Negro and White Races, with Its Relation to Clinical Coronary Artery Disease, Arch. Path. **50**:189 (Aug.) 1950.

2. Lansing, A. I.; Alex, M., and Rosenthal, T. B.: J. Gerontol. **5**:211, 1950. Lansing, A. I.; Roberts, E.; Ramasarma, G. B.; Rosenthal, T. B., and Alex, M.: Proc. Soc. Exper. Biol. & Med. **76**:714, 1951.

the administration of lipids. Certain as yet unpublished observations [3] indicate that wear-and-tear factors, as a function of time (age), are important in determining the rate of development of arteriosclerosis. In a previous report it was noted that the rate at which aging processes develop in the coronary arteries in Negroes lags behind that in white individuals.[1d] Pertinent clinical findings have shown that in Negroes there is a greater incidence of arterial hypertension but less disease of the coronary arteries and a lower autopsy incidence of coronary thrombosis. Furthermore, in the latter investigation the rate of breakdown of the internal elastic lamella appeared to

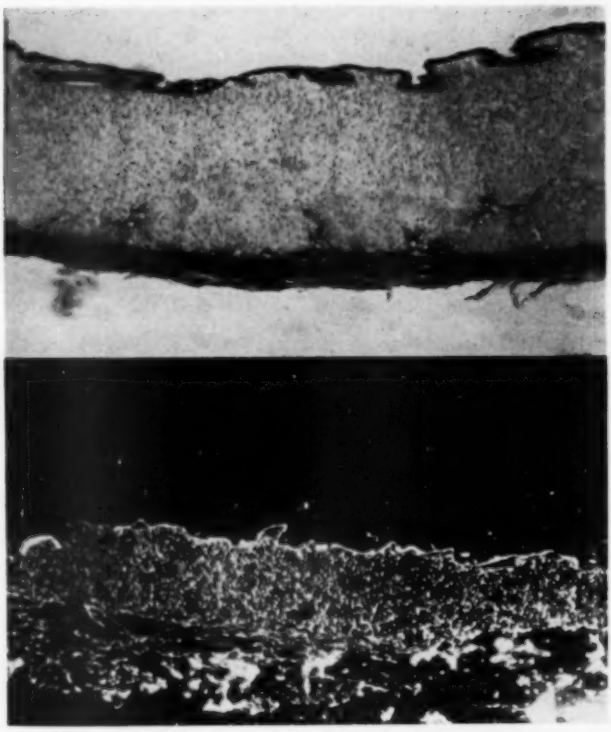

Fig. 1.—Sections of the wall of the middle third of the renal artery of a 19-year-old white man. Upper part: The external elastic lamella is thick; elastic elements are beginning to extend into the outer media. Weigert-Verhoeff stain; × 100. Lower part: The microincinerated specimen shows a fine calcium line corresponding to the inner elastic lamella. Dark-field illumination of microincinerated specimen; × 100.

represent an influencing factor in determining plaque formation. Therefore, the present method of study also affords a means of determining anatomical manifestations of racial difference.

The present report represents a continuation of the studies noted above, but deals specifically with the aging changes in the renal and the splenic arteries of Negro and

3. Blumenthal, H. T.; Handler, F. P., and Blache, J. O.: To be published.

white persons; it was designed (1) to determine whether or not racial influences as established in the coronary arteries exist in these vessels; (2) to study the severity of degenerative changes as related to disease, and (3) to compare the splenic artery with the major human arteries already reported on, with respect to rate and pattern of aging. Such a comparison was thought to be especially important because of the known sensitivity of the splenic artery to epinephrine.

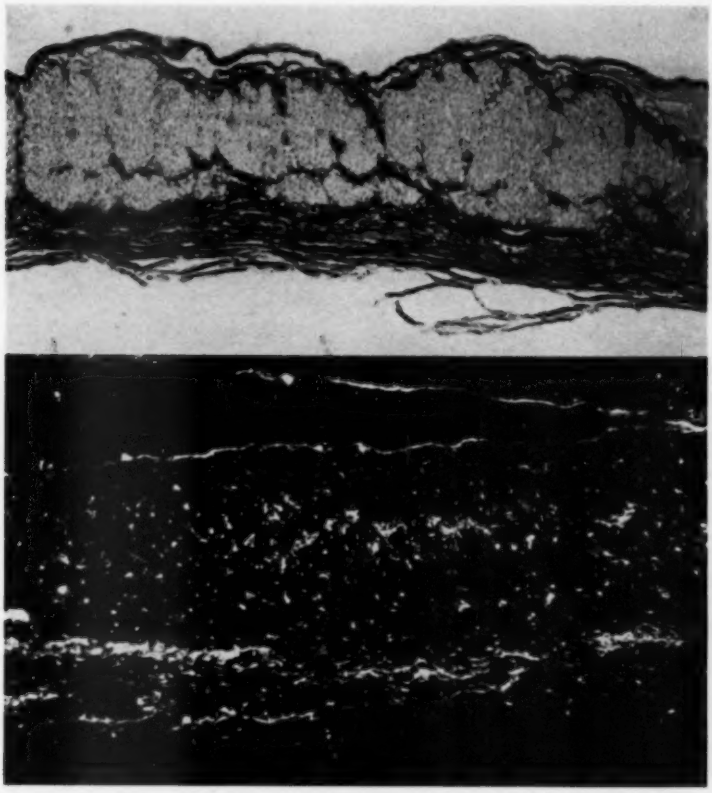

Fig. 2.—Sections of the wall of the middle third of the renal artery of a 21-year-old Negro man. Upper part: The internal elastica is frayed, and long fibrils migrate into the media. Fibrils from the internal elastica are present in the upper portion of the media. Weigert-Verhoeff stain; × 100. Lower part: Microincineration reveals 2+ calcium deposition in the outer media and the external elastica. Dark-field illumination of microincinerated specimen; × 100.

MATERIAL AND METHOD

The material utilized in this study consisted of 399 arteries of 208 patients, the anatomical, racial, and age distribution of which are shown in the accompanying table. Each specimen consisted of approximately the distal 1 cm. of the splenic or the

renal artery fixed in formaldehyde solution U. S. P. diluted 1:10 in dehydrated alcohol N. F. Sections were prepared by methods previously reported.[4]

RESULTS

A Comparison of Aging Processes in Renal Arteries from Negro and White Individuals

Age Group 0 to 30 Years.—During the first decade the renal arteries of Negro and white persons are similar in appearance. The endothelium is a unicellular layer resting on an intact wavy internal elastic lamella. The external elastic fibers are compact and form three or four thick parallel bands. The media is free of elastic fibrils. Except for a slight accentuation of a thin calcium line along the inner surface of the vessel, mineral deposition is nuclear throughout.

In the succeeding decades in both races of this age group the endothelial layer remains unchanged. Subendothelial proliferation, evidenced by the presence of fibroblasts and loose collagen strands, occasionally occupies as much as one-eighth of the entire thickness of the vessel wall. In the arteries of Negro patients the internal lamella begins to straighten but for the most part remains a well-defined intact band. However, the elastic fibers of the external lamella are separate, and large thick strands penetrate into the media; small fibrils are seen breaking from

Distribution of Cases

Age Group	Splenic Artery		Renal Artery	
	Negro	White	Negro	White
0-30 yr.	9	16	6	16
31-50 yr.	22	22	20	24
51-70 yr.	22	62	20	61
71 yr. and over	8	39	6	46
Total	61	139	52	147

these strands. Occasionally the internal elastica shows small foci of fraying, with thin slivers of elastic tissue entering the adjacent media, and frequently there is an intermingling of fibrils from the two lamellae. Elastic filaments are sparse in the media and, when present, are usually in the neighborhood of the large fragments. Calcium deposition occurs along the fibrils and in patchy areas along the external elastica, averaging 1.5+. A patchy granular pattern of calcium is occasionally seen associated with elastic tissue fragments in the outer half of the media.

The renal arteries of white persons show essentially the same pattern, with the exception of certain changes along the external lamella. Some fine fibrils split from the external lamella, but thick, large fragments, which constitute the common pattern in Negroes, are rarely encountered in the renal arteries of white persons. The elastic fibers of the external lamella are short and often broken. Nuclear calcium deposition is present in the media; calcification along the external lamella is generally less than in the Negro, averaging 1+. No intimal atheromata have been encountered in either racial group (Figs. 1 and 2).

Age Group 31 to 50 Years.—On the average, diffuse subendothelial thickening of the type observed in the previous two decades occupies 10% of the thickness of the arterial wall in both races; the endothelium remains in a well-defined unicellular

4. Lansing and others.[1b] Blumenthal and others.[1c] Blache and Handler.[1d]

layer. Even in the fourth decade of life occasional arteries show essentially negligible subendothelial proliferation. In all instances fine, small fibrils arise from both elastic lamellae and penetrate the adjacent media (Fig. 3). Fraying and proliferation of the internal lamella, producing a pattern similar to that described in the coronary artery, are considerably more pronounced in the Negro examples. Foci of intense calcification with deposition of bony lamellae are noted in one Negro renal artery,

Fig. 3.—Section of the wall of the middle third of the renal artery of a 35-year-old Negro man. Upper part: The external elastica shows patchy fraying. The fragments extend into the outer half of the media; there is no subintimal thickening; Weigert-Verhoeff stain; × 100. Lower part: Microincineration reveals 2+ calcium duplication of elastic fragments in the media. Dark-field illumination of microincinerated specimen; × 100.

while none has been found in the white group. In addition, in vessels of Negroes, large fragments are seen jutting into the media from the external elastica. In the latter race there are also separation and swelling of individual strands of elastic tissue of the external lamellar zone; thus wide bundles of long, plump fibers are

formed, which spread into the adventitia. The external lamellae of the renal arteries of white persons continue to contrast sharply with those of Negroes. The calcification along the external lamella and the outer part of the media corresponds to the anatomical location of these elastic elements and averages less than 1.5+ in the white race, compared with approximately 2.5+ in the Negro race. These racial differences are illustrated in Figures 4 to 8, which pertain to a subsequent age group where they become more accentuated.

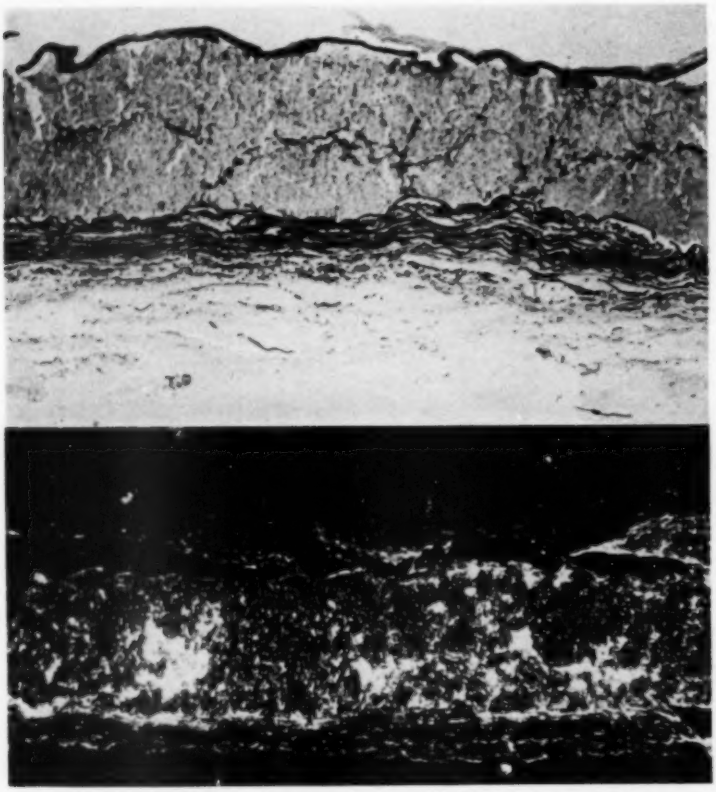

Fig. 4.—Section of the wall of the middle third of the renal artery of a 53-year-old Negro man. Upper part: Fragmentation of the external elastica is moderate; fibrils are seen ramifying through the media; Weigert-Verhoeff stain; × 100. Lower part: Microincineration reveals 2.5 calcium deposition, occurring in the external lamella and duplicating fragments in the media. Dark-field illumination of microincinerated specimen; × 100.

Whenever diffuse subendothelial thickening is noted, the internal elastica shows fraying and fragmentation, with elastic fibrils projecting into the collagen of the subintima. In both races such foci show a similar mineral pattern, in which calcium averages 1+. The most extensive foci of fraying of the internal elastica lamella of the Negro renal artery occurred in 2 of the 20 specimens, and this process was

associated with thick eccentric collagen plaques containing only slight lipid deposits. No intimal lipid plaques were found in the 24 renal arteries from white persons.

Age Group 51 to 70 Years.—In this group there is no change in the endothelial layer. The subendothelial fibrous tissue layer averages 20% of the thickness of the arterial wall; in occasional vessels atheromatous plaques are encountered, which may

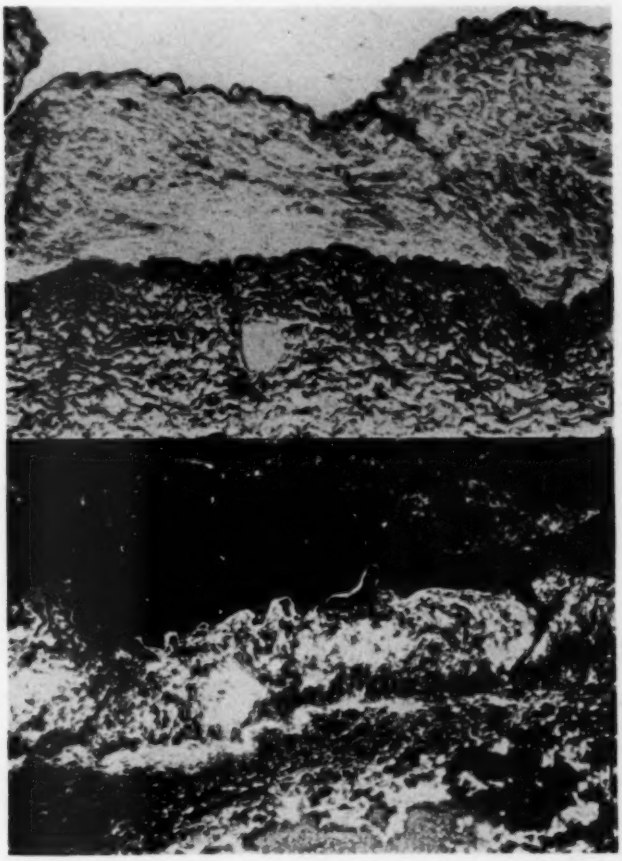

Fig. 5.—Section of the wall of the middle third of the renal artery of a 62-year-old white man. Upper part: Marked elastic proliferation of the external elastic lamella and numerous elastic extensions into the media may be seen. The inner elastic bundle remains as a single band in most areas. Weigert-Verhoeff stain; × 100. Lower part: Microincineration reveals intense calcification of elastic elements in the media and of the external elastic lamella. The inner elastic lamella can still be identified as a fine line of calcium. Dark-field illumination of microincinerated specimen; × 100.

constitute as much as 50% of the vessel thickness in focal areas. This occurred in 5 of the 20 Negroes and in 5 of the 61 white persons. In the arteries of members

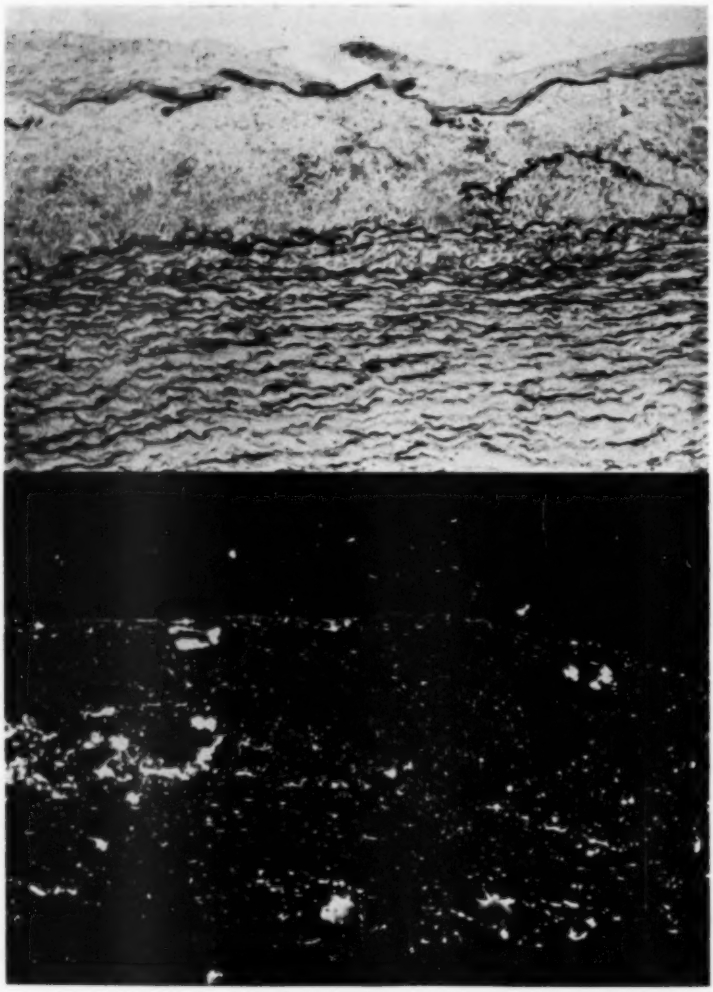

Fig. 6.—Section of the wall of the middle third of the renal artery of a 63-year-old Negro man. Upper part: The internal lamella is extensively frayed and broken; well demarcated dense granular foci are present; subintimal thickening occupies 15 to 20% of the wall. Weigert-Verhoeff stain; × 100. Lower part: microincineration reveals 4+ calcification duplicating the granular areas of the internal lamella. Dark-field illumination of microincinerated specimen; × 100.

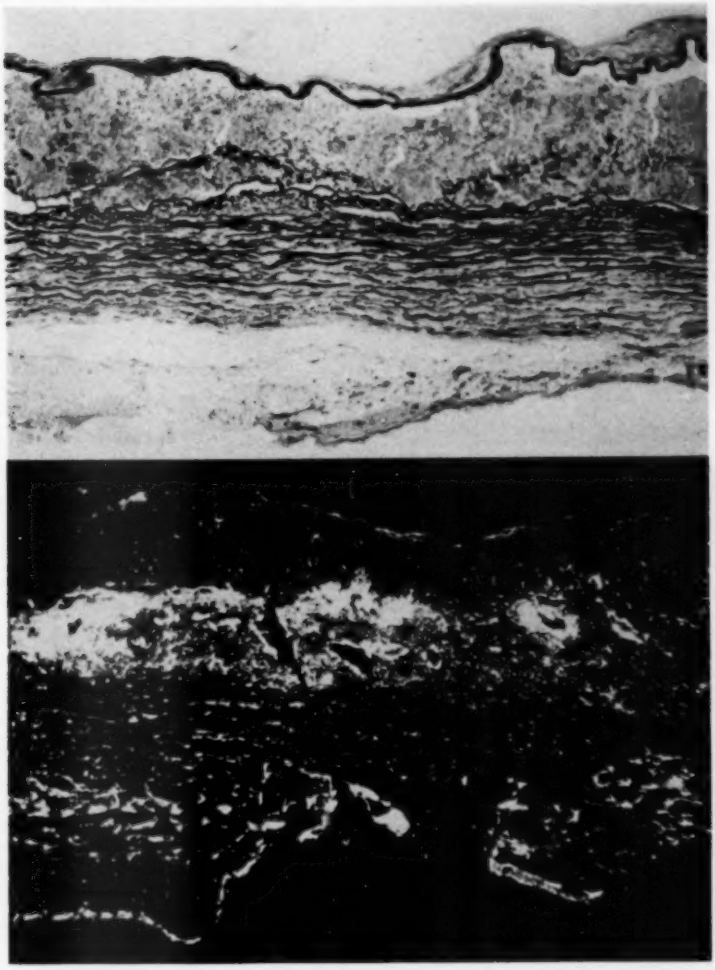

Fig. 7.—Section of the wall of the middle third of the renal artery of a 64-year-old Negro man. Upper part: The external lamella shows marked fragmentation; fibrils are plentiful in the media; the internal lamella is straightened, thickened and frayed. Wiegert-Verhoeff stain; × 100. Lower part: Microincineration reveals calcium deposition to be 4+ in the outer media and along portions of the external lamella. Dark-field illumination of microincinerated specimen; × 100.

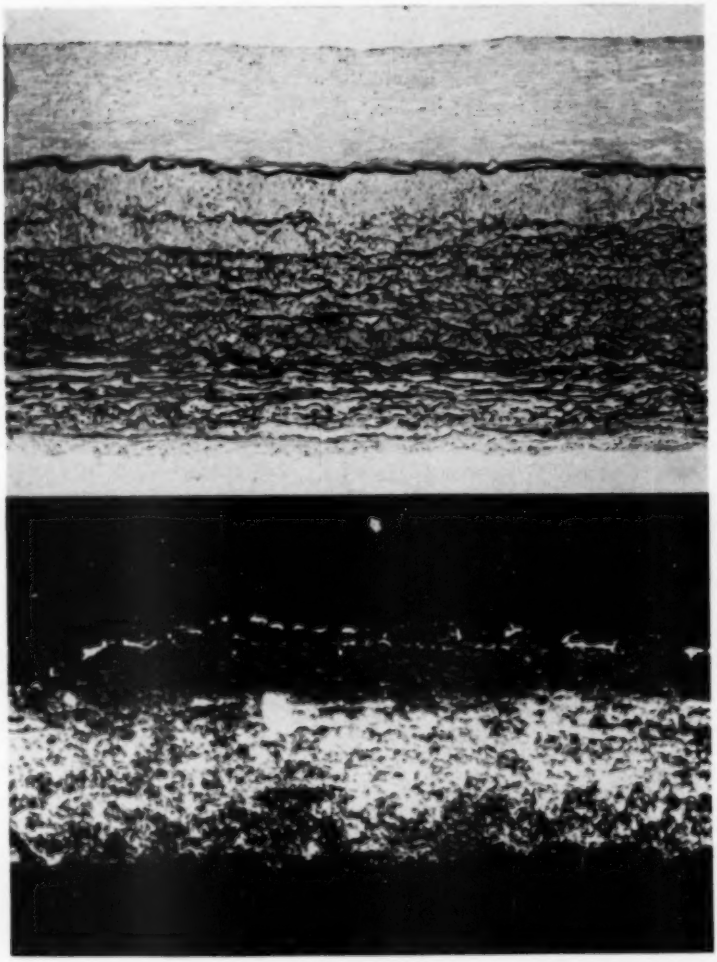

Fig. 8.—Section of the wall of the middle third of the renal artery of a 68-year-old Negro man. Upper part: Fraying of the external elastica is severe with parallel fibers penetrating to the internal lamella; subintimal thickening occupies 30% of the vessel wall; the internal lamella is straightened and broken. Weigert-Verhoeff stain; × 100. Lower part: Microincineration reveals a 3 to 4+ calcification duplicating the fibers in the media. Dark-field illumination of microincinerated specimen; × 100.

of both races the internal elastica lamella has lost much of its undulating quality and is present as a straightened band with frayed edges. From one edge filaments enter the subendothelial tissue; the intensity of this change is directly proportional to the thickness of the subendothelial layer and to the deposition of calcium along elastic fibrils. The subendothelial layer is somewhat thicker in Negro specimens, and the fraying and spreading of elastic fibrils are more intense than in white ones. Calcification along this layer averages about 1.5+ in the white race and 2+ in Negroes. In addition, three of the Negro specimens show well-defined calcareous deposits within the internal elastic lamella resembling bone, while none has been found in the white group.

Elastic extensions into the media from both lamellae become progressively more evident in this age group. More fine filaments are formed, and these progressively

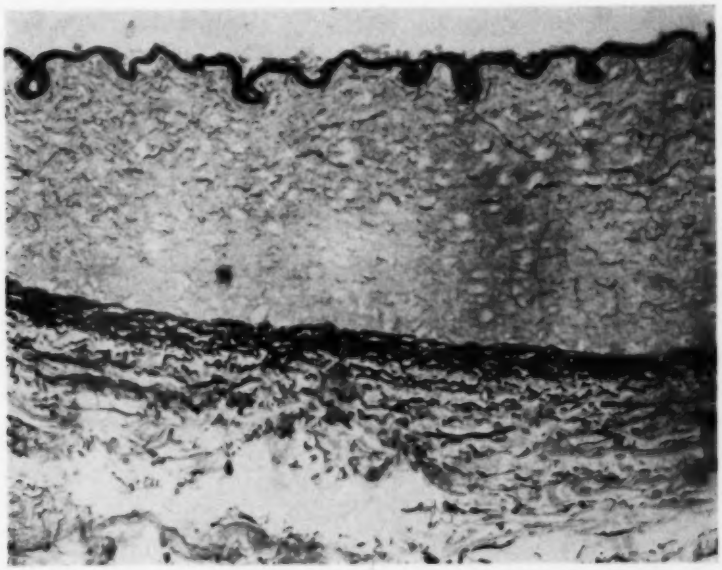

Fig. 9.—Section of the wall of the splenic artery of a 12-year-old white girl. The inner surface is a single layer of endothelial cells with undulations of internal elastica and nuclear deposits of calcium. Elastic filaments are present in the inner half of the media; these disappear in older specimens. Weigert-Verhoeff stain; × 250

break down into smaller and smaller particles until foci of fine granular material with an affinity for the elastica stain become apparent. The arteries of individuals of the white race consistently have far fewer coarse elastic fragments in the media, though small filaments and granular material are plentiful. The external elastica, therefore, continues to show the sharp contrast of shorter, narrower fibers held in compact bands as compared with the long, thick, splinter-like fragments present in the arteries of Negro patients. Calcification along such areas averages 2.5+ in the white group, compared with 3+ in Negroes. Foci of 4+ calcification occur in both groups but are commoner in the Negro group (Figs. 4 to 7). In several white

persons but in only one Negro, a pattern of elastic fibers similar to that seen in the aorta or the iliac artery was noted in the media of the renal artery (Fig. 8).

Age Group 71 Years and Over.—In the renal arteries of this age group racial differences become less distinct. There is no change in the endothelial line, but the subendothelial zone averages about 40% of the thickness of the vessel wall. Plaques containing cholesterol slits, and mineral deposits, are recognized readily in hematoxylin-eosin preparations of 2 of the 6 Negroes arteries and 6 of 46 white ones. The internal elastic lamella is now quite straight and shows greatly increased fraying along both margins; numerous elastic fibrils which seem to be derived from these frayed edges penetrate the media and the intima. There is apparently a subtle difference between the internal elastic lamellae of white and Negro renal arteries, since in one specimen of the latter group foci of apparent bone formation are noted within the internal elastic lamella, similar to that already described in previous decades; this phenomenon has not been observed in any of the arteries from white patients. Other than this, calcific changes are similar in the two races; the average calcification along the inner elastic lamella and along the elastic filaments in the subintima is about 2+ and that along the external lamellar zone and along its intramedial elastic extensions, apparently derived from the latter, is 3 to 4+.

A Comparison of Aging Processes in Splenic Arteries from Negro and White Individuals

Age Group 0 to 30 Years.—As in the case of the renal artery, the inner surface of the splenic artery consists of a single layer of endothelial cells. The internal elastic lamella lies in contact with this lining and shows numerous undulations. During the latter half of the first decade fine elastic filaments appear in the inner half of the media, which appear to be derived from the internal elastic lamella, but the outer half of the media is devoid of elastic elements (Fig. 9). There is a thin line of calcium along the inner surface of the vessel, and calcium distribution is nuclear throughout the media. The external lamella consists of four to five rows of elongated, fairly plump elastic fibers. No racial difference is noted.

During the following two decades, subendothelial fibroblasts and collagen appear and separate the endothelium from the internal elastic lamella; this subendothelial layer becomes progressively thicker until, in the third decade, it occupies 5 to 10% of the thickness of the arterial wall. The internal elastic lamella also progressively thickens and loses some of its undulations; the surface bordering on the subendothelial layer begins to fray, and early calcium deposition is noted along such filaments. Some calcium is also deposited along the frayed surface of the internal lamella bordering on the media. In addition, there is slight precipitation of calcium in foci in the outer half of the media which appears to be independent of any elastic elements. During the third decade some of these areas show 1+ calcification. The external lamella consists of 4 to 12 parallel layers of plump, short elastic fibers which do not significantly penetrate the outer media. The intensity of these changes is not significantly different in the two races, nor is there any noteworthy sex difference. No intimal lipid-containing plaques have been found in either racial group. These features are illustrated in Figure 10.

Age Group 31 to 50 Years.—The endothelial lining remains unchanged. The subendothelial fibrous layer progressively thickens, averaging about 10 to 15% of

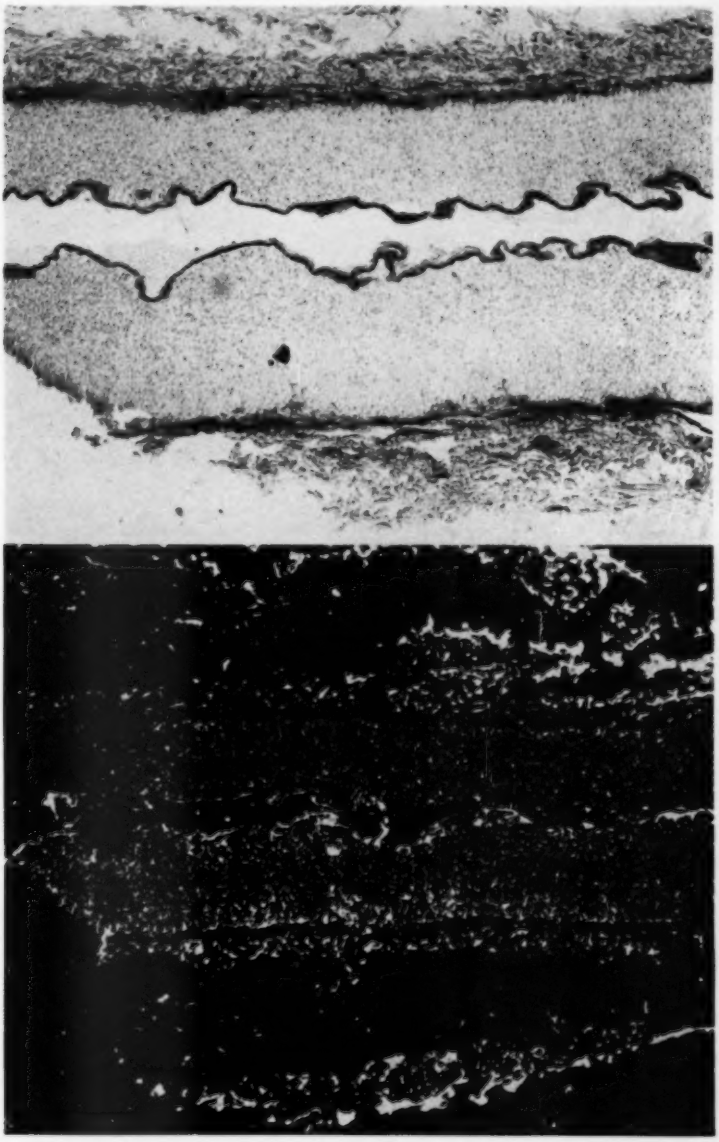

Fig. 10.—Section of the wall of the splenic artery of a 29-year-old Negro woman. Upper part: The internal elastic lamella shows numerous undulations. Weigert-Verhoeff stain; × 110. Lower part: Microincineration shows a thin line of calcium along the inner surface. Dark-field illumination of microincinerated specimen; × 110.

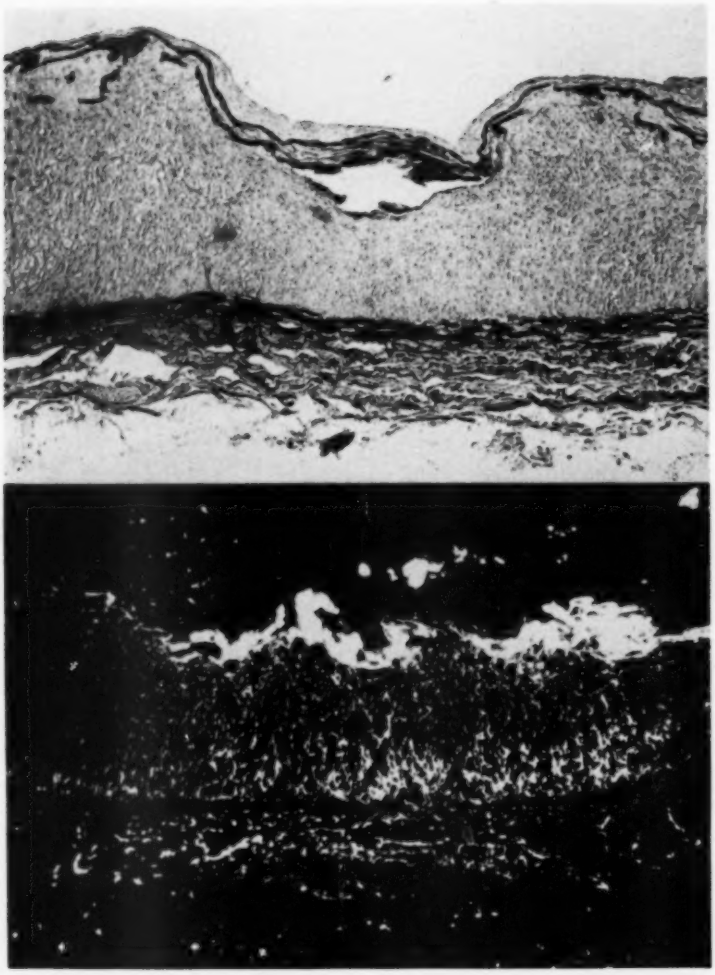

Fig. 11.—Sections of the wall of the splenic artery of a 46-year-old Negro man. Upper part: There is a diffuse subendothelial fibrous thickening. The tear at the base of the elastic mass in the center represents an area of bone formation. The media remains free of elastic elements. Weigert-Verhoeff stain; × 110. Lower part: Microincineration reveals dense calcification of intimal elastic masses. Dark-field illumination of microincinerated specimen; × 110.

the thickness of the arterial wall; focal areas occupy as much as 30% of the wall, but in occasional cases no significant subendothelial layer is shown. Lipid-containing plaques are found in only one case in each racial group. Elastic filaments extending from the internal elastic lamella into the subendothelial tissue and many foci of fraying of this elastic band along its intimal margin are noted; the intensity of such changes appears to be in direct proportion to the degree of subendothelial thickening. Foci of increased density of the internal lamella are also noted, and in such areas there is complete loss of the undulating quality. The medial side of the internal elastica also shows fraying, and the inner half of the media shows moderate extension of the elastic filaments. The areas of increased granular density of the internal elastic band show 3 to 4+ calcification, and in both races areas of apparent bone formation similar to that noted in several of the renal arteries of the Negro group are noted (Figs. 11 and 12). The average calcification along the internal elastic lamella is 1.3+ in both races, and there is only an average 0.5+ calcium deposition along elastic filaments in the inner half of the media. In the outer half of the media, focal deposits of calcium, apparently not associated with elastic elements, average 1.8+ in white and 1.4+ in Negro specimens; in one artery from a Negro who had arterial hypertension this had gone on to bone formation (Fig. 12). There is no appreciable change in the external lamella other than an occasional extension of elastic filaments from this layer into the outer media, which contributes some calcification to the latter area. However, a network of joining filaments derived from the two elastic lamellae such as was frequently noted in renal arteries does not occur in the splenic arteries.

Age Group 51 to 70 Years.—In the splenic arteries of both races the subendothelial fibrous thickening shows only a slight increase over that noted in the previous age group, averaging about 15% of the thickness of the wall. Lipid-containing plaques are found in 12 white and 6 Negro specimens (18 and 27%, respectively). Beneath such plaques there are collections of elastic filaments apparently derived from the internal elastic lamella and foci of marked fraying of the latter (Fig. 13). Calcification along the internal lamella averages 1.3+ in the Negro and 1.6+ in the white specimens (18 and 24%, respectively). In this as well as in the previous age group foci of intense calcification occur in association with gaps in the internal elastic lamella across which a few fine elastic filaments stretch. Scattered elastic filaments are distributed through the inner half of the media and show only mild calcification, averaging about 0.5+. There is an increase in the intensity of granular calcium deposition in the outer half of the media; this averages about 1.5+ in both races. In specimens from three white patients there is bone formation in the outer half of the media apparently not associated with any elastic tissue change; two of these arteries are from diabetic patients and the third is from a patient with long-standing hypertension. The external lamella shows no appreciable alteration except that in an increased number of cases there are a thinning-out and an apparent diminution in the number of elastic fibers in this zone.

Age Group 71 and Over.—In both races only occasionally does a subendothelial fibrous layer occupy less than 10% of the thickness of the wall of the splenic artery; the average thickness is about 20%. The internal elastic lamella appears straight, rigid, thick, and dense but shows foci of advanced fraying as well as of elastic tissue proliferation along its internal surface. Gaps are noted as in some specimens in

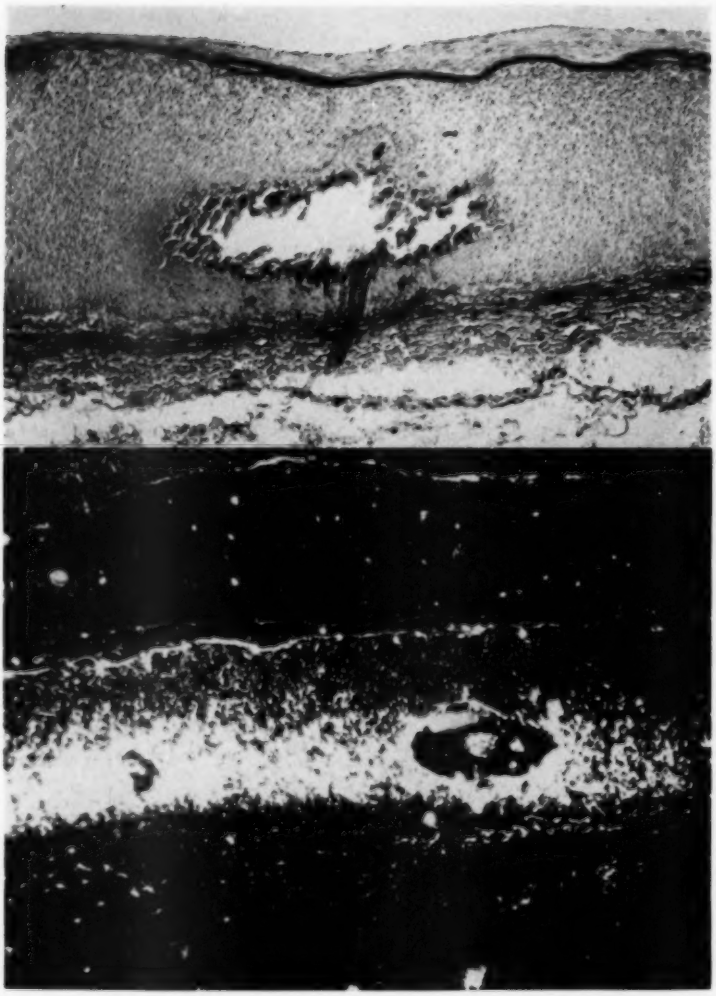

Fig. 12.—Sections of the wall of the splenic artery of a 49-year-old hypertensive Negro man. Upper part: The endothelial lining is unchanged, but there is straightening of undulations of the internal elastica and fibrous plaque formation. The tear in the media represents an area of dense calcification not associated with elastic elements. Weigert-Verhoeff stain; × 110. Lower part: Microincineration shows intense foci of calcification with bony lamellae in the outer half of the media and some intensification of the internal elastica. Dark-field illumination of microincinerated specimen; × 110.

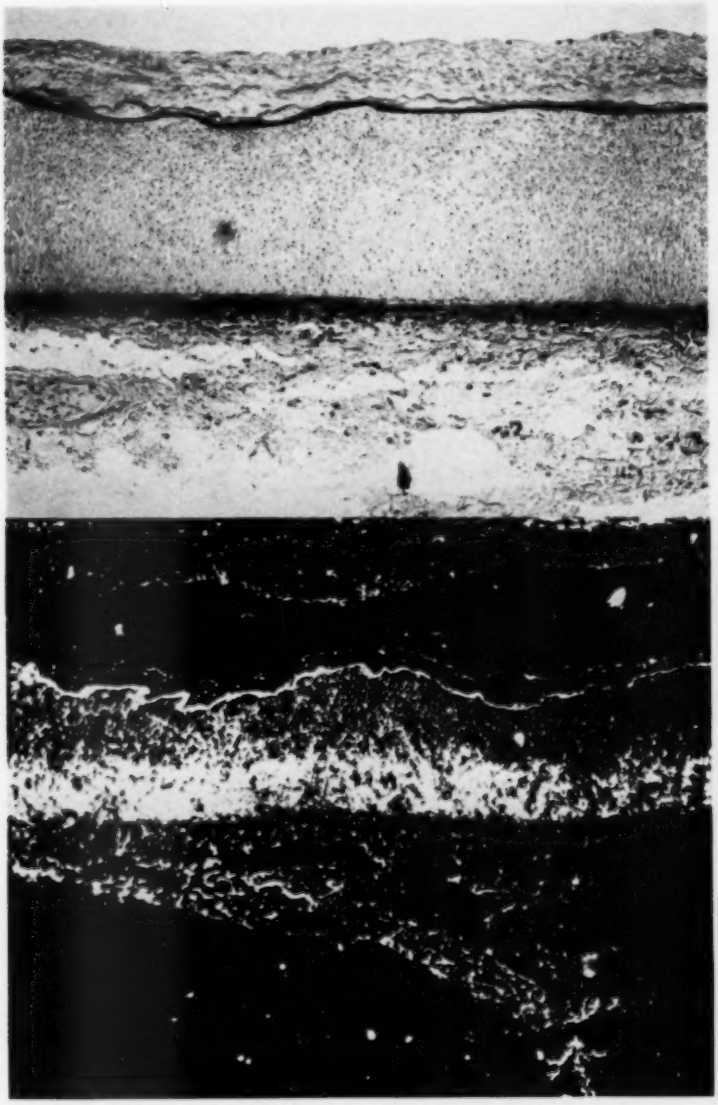

Fig. 13.—Sections of the wall of the splenic artery of a 58-year-old Negro man. Upper part: There is marked intimal thickening. The internal elastic lamella has lost its undulations, and fine granular material is present in the intima. The media is free of elastic elements. Weigert-Verhoeff stain; × 110. Lower part: Microincineration reveals 4 + calcification in the outer half of the media and fairly marked accentuation of the internal lamella. Dark-field illumination of microincinerated specimen; × 110.

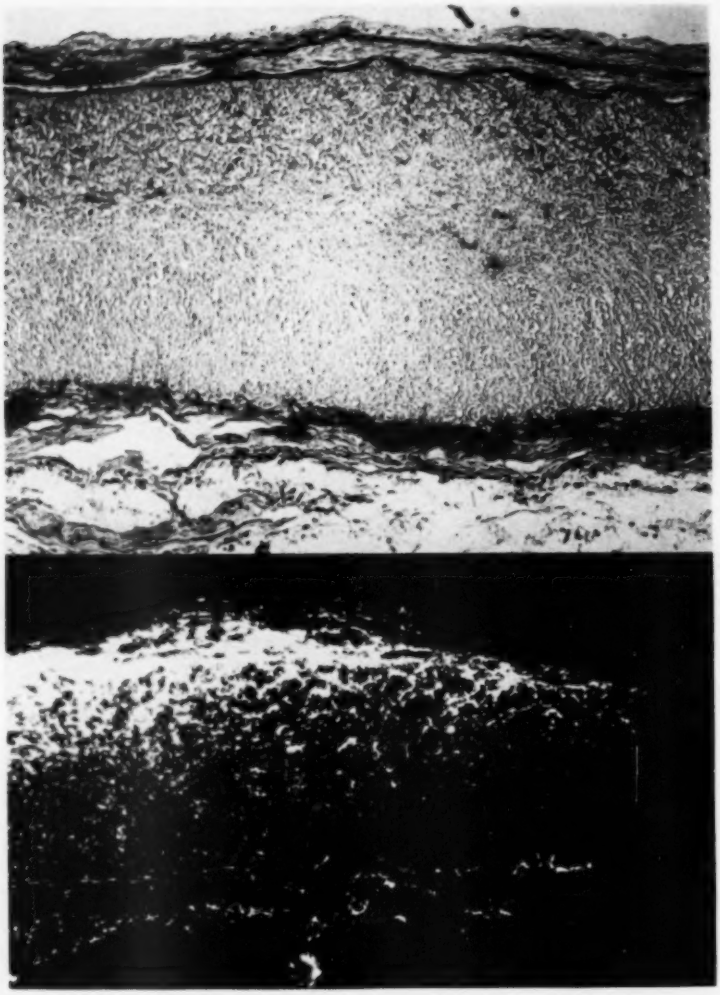

Fig. 14.—Sections of the wall of the splenic artery of an 89-year-old white woman. Upper part: The internal elastic lamella shows traying and duplication. The elastic fibrils from the edges penetrate into the intima and media. Weigert-Verhoeff stain; × 110. Lower part: Microincineration reveals 2+ calcification of the internal elastic fibers and of fibrils penetrating into the intima and media. Dark-field illumination of microincinerated specimens; × 110.

several previous decades, and bone formation along the internal elastic lamella is frequently encountered. The latter is found in 5 of the 8 Negro specimens (63%) and 14 of the 38 from white patients (37%). However, the degree of calcification along the internal elastic lamella averages 1.5+ in Negroes and 2.0+ in the white group. Scattered elastic filaments are present in the inner half of the media in both races, showing an intensity of calcification not exceeding 1+. In the outer half of the media, however, the average intensity of focal calcification is 1.1+ in both races, and this bears no relationship to elastic elements. The external lamella does not change perceptibly over the previous several decades and shows only occasional foci of 1+ calcification.

The Relation of Disease to the Intensity of Aging Processes

With respect to the effects of hypertension, as stated in a previous report,[1c] the renal arteries of white patients appear to show less calcium deposition and a more moderate degree of elastic tissue alteration only when the patients are past 80 years of age; the same differences are also present in white patients with cancer. In other age groups of the white series these diseases do not appear to play a significant role in the rate of renal arterial aging. There is only one white patient with diabetes, aged 65, whose renal artery shows 3.5+ calcification along the external lamella, compared with an average of 2.6+ for the group composed of white persons in the seventh decade of life; no significance is attached to this finding in a single case.

In the Negro group, hypertensive patients between ages 51 and 70 show a slightly greater intensity of calcification of the internal elastic lamella (2.5+ in the hypertensive, compared with 2.0+ in the nonhypertensive ones), but the other portions of the wall of the renal artery show no discernible difference. There is no significant difference between cancer and noncancer patients in any age group. Comparison of the effect of hypertension as with white patients in the groups over age 71 is not possible, since there are only 7 Negroes in this category.

The data do not indicate any significant effect of tuberculosis on the rate or the intensity of these changes in either racial group.

COMMENT

In contrast with the present trend to determine the role of general metabolic factors in the genesis of arteriosclerosis, our investigations are designed to deal with factors which may explain the focal nature of this disease. In general, these focal characteristics fall into two categories:

1. There is a predilection of arteriosclerosis for certain vessels, such as the aorta and the cerebral and coronary arteries, as well as the arterial trees of the lower extremities. An understanding of the factors determining this distribution may also explain the definite immunity from lipid plaque formation of the pulmonary arterial system as well as of the small branches of arteries and arterioles within parenchymatous organs, and the lower degrees of immunity of the hepatic, splenic, and renal arteries.

2. There is also a predilection of sites of plaque formation for certain portions of arteries. For example, there is frequency of involvement of the first several centimeters of the coronary arteries, with relative sparing of the more peripheral portions of the coronary arterial system. Similarly, sclerosis of the aorta is much severer

in the abdominal than in the thoracic portion, while in syphilitic aortitis the reverse occurs; furthermore, within the aorta there is a distinct predilection for plaque formation on the posterior surface and at the sites where branches originate.

Such localizing phenomena are not well duplicated in the experimental animal subjected to various procedures designed to alter lipid metabolism. Furthermore, there appears to be some local factor, intrinsic either in structure or in metabolism, which determines these sites of predilection, since in hypertension which may be due to circulatory factors probably hormonal in character, the formation of arteriosclerotic lesions may be intensified, but the focal character of distribution remains essentially the same.

From the foregoing statements it seems appropriate that the initial phase of investigations dealing with factors which may determine the focal nature of arteriosclerosis should be a careful charting of anatomical and histochemical differences of various major arteries and their branches, as well as of arterioles, in an attempt to correlate such differences with susceptibility to this disease. Our introductory remarks indicate previous progress along such lines. The method utilized in these investigations is not original with us, as pointed out in previous reports,[1] but an organized approach to this problem has not been made heretofore. Previous material combined with that in the present report indicates that the elastic tissue pattern of arteries is not fixed but shows age changes which vary considerably in different blood vessels. Further, the elastic structures show a striking affinity for calcium, and the deposition of this mineral along elastic fibers increases with advancing age. This affinity for calcium has also been observed by several other investigators,[5] in some instances in unrelated investigations.[5d]

It was initially shown in our investigations that the calcium which is progressively deposited in the media of the aorta as a function of age disappears in large part when elastic fibers are destroyed, as in syphilitic aortitis.[1a] Recent observations on this vessel, as yet unpublished,[3] have shown a variability in the pattern of elastic tissue-calcium change of the internal elastic lamella which may be correlated with certain physical factors and with the subsequent localization of intimal plaques. The aging pattern in the iliac artery is essentially similar to that observed in the abdominal aorta.[1c]

Further, it has been demonstrated that in the coronary artery [1b] it is the internal elastic lamella which loses its identity through fraying and the laying down of elastic fibrils and filaments along with progressive calcification of these elements. Such changes are particularly notable at the base of lipid-containing plaques but may also be seen in less degrees in other areas where lipids are either absent or just beginning to become apparent. Pertinent to such observations are the data derived from the previously mentioned studies of coronary artery aging in the Negro race,[1d] in which it was shown that the incidence of coronary thrombosis is considerably lower in Negro than in white persons and also that the rate of development of these elastic tissue–calcium changes is considerably slower in the former. The hepatic artery, which thus far has been studied only in the cases of white persons, presents a

5. (*a*) Bittrolf, R.: Beitr. path. Anat. **49**:213, 1910. (*b*) Ravault, P.: Bull. histol. appliq. à la physiol. **6**:49, 1929. (*c*) Zinkant, W.: Arch. path. Anat. **281**:911, 1931. (*d*) Harris, P. N., and Wulzen, R. M.: Am. J. Path. **26**:595, 1950.

pattern of aging similar to that observed in the coronary artery but developing at a much slower rate, and, correspondingly, lipid plaques occur with considerably less frequency.[1e]

In the hepatic, coronary, and splenic arteries the external elastic lamella shows remarkably little alteration with age, while in the renal artery this elastic layer shows extensive calcification as described here and in a previous report. On the other hand, the internal elastic lamellae of the splenic and renal arteries show a similar aging pattern, which develops, however, with greater intensity in the splenic vessel. Again fraying of the internal elastic lamella, with deposition of elastic filaments and fibrils and calcification of these elements, occurs most intensely under lipid-containing plaques, and, as noted previously in other vessels, forms the source of calcium commonly seen in these intimal elevations.[1e] Again, in areas where lipid deposition is not a striking feature accompanying subendothelial fibrous thickening, these changes appear to represent an early stage of plaque formation. The latter changes are focal in character, but, in addition, there is a general progressive straightening, thickening, and calcification of the internal elastic lamella in splenic and renal arteries over wide areas, which progresses in many instances to actual bone formation and probably represents one type of calcification of arterial walls included under the term "Mönckeberg's sclerosis." Another type of progressive calcium deposition occurs characteristically in the outer half of the media of the splenic artery and may also go on to bone formation. It does not appear to be associated with changes in elastic elements. It has been noted thus far only in a few hypertensive patients and in two diabetic patients; whether or not it occurs in other conditions remains to be determined. Thus we perceive that the term Mönckeberg's sclerosis includes two different processes, one involving calcification of elastic tissue and one in which calcium is deposited in areas devoid of elastic elements.

Contrary to the findings in the coronary arteries, the renal arteries appear to age more rapidly in Negro than in white patients with respect to changes in both the internal and the external elastic lamellae, and this is probably related to the higher incidence of hypertension which has been reported in the Negro. However, these findings along with the more intense calcification of the aorta and the splenic artery in relatively young white hypertensive persons, probably represent an effect of this disease rather than an etiological factor.

As pointed out in a previous report,[1e] blood vessels have generally been considered as more or less fixed tubular structures in a physical sense, with the exception that in them atheromatous plaques may develop which are believed to be due to a filtering of lipids through the endothelial lining. Instead, we would consider the vascular structures of the organism as a highly specialized organ system with variable quantities of component elements; these include endothelium, elastic tissue, smooth muscle, fibrous elements, and "ground" or cement substance. The variability in component elements is probably related to specific function. Thus the aorta and the pulmonary arteries contain a high proportion of elastic elements to cope with large hydrostatic tensions and possibly to facilitate the transmission of the pulse waves, while other arteries, exemplified by the coronary and splenic vessels, contain elastic elements in smaller amounts to cope with somewhat lower hydrostatic tensions and a greater proportion of smooth muscle to produce the necessary constrictive forces.

This organ system, in common with others, contains its own enzymes [6] as well as vascular and lymphatic channels.[7] Furthermore, it has been recently demonstrated that the aorta is capable of synthesizing cholesterol.[8] In considering such a concept of the arterial wall, it is possible, as we have previously pointed out,[1c] that intimal lipids may originate as a product of metabolic processes within the vessel wall. In order to establish the origin of plaques as being due to lipids infiltrating from the serum, emphasis has been placed on elevated serum lipid levels by many investigators or on an increase in certain types of circulating lipoproteins.[9] The blood is not a living tissue in the same sense as other tissues of the body. It is primarily a transport mechanism, and the level of any substance in the blood is a reflection of metabolic processes in tissues. Such levels are determined by the balance between the synthetic processes and the processes of breakdown. The blood vessel wall, on the other hand, is a tissue with metabolic processes like other organs; since it is in intimate contact with the circulating blood, it may be contributing to the blood lipids and high circulating lipid levels may, therefore, represent an effect rather than a cause of arteriosclerosis. That the endothelium is permeable for large lipid molecules has not been established by direct observation, and the theory that the lipids are brought into the intima by lipophages has largely been abandoned. On the other hand, those reports dealing with elevated lipid levels and lipoprotein fractions in human cases [9] have not cited values approaching the high levels produced in experimental animals, in which xanthoma-like masses are found in several organs, such as the liver and the spleen; the latter are rarely observed in humans even in those with extensive arteriosclerosis. Therefore if lipids play a role in the genesis of arteriosclerosis, it seems likely that this is on a local level within the vascular wall rather than on the basis of generalized metabolic disturbances.

Histological observations concerning the sequence of events in the formation of atheromatous plaques are also pertinent to this discussion of the mechanism of arteriosclerosis. The present results show that there is a progressive symmetrical fibrous thickening of the subendothelial tissue as part of the aging process. In general it remains symmetrical about the inner circumference of the vessel wall for approximately the first three to four decades of life, after which asymmetrical foci of further thickening are noted to which the elastic elements contribute. It is only after this has taken place that lipids appear in notable amounts, and they are located, as a rule, not near the surface, but deep in the intimal layer, adjacent to calcifying elastic elements. These processes occur in varying intensities and apparently at varying rates in all arteries thus far studied; when they occur at a slow rate, as in the case of the hepatic artery, lipid plaque formation and thrombosis are infrequent occurrences. Such observations on vessels in an age series tend to confirm data

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presented by Lansing and co-workers [9] and by Yater and co-workers [10] that fibrous plaques precede the formation of atheromata.

The methods of investigations used here deal primarily with the elastic tissue–calcium changes occurring with age, but these alterations are regarded as only representative of changes occurring in other elements of the arterial wall. Degenerative changes occur in muscle, fibrous tissue, and "ground" or "cement substance"; it has been shown that lipids, as well as metachromatically stainable carbohydrate–sulfuric acid esters, increase with age in the arterial wall.[11] We have centered our efforts on elastic tissue and calcium because they are easily traced and readily identified. The calcification which occurs in the outer half of the media of the splenic artery probably represents a progressive degenerative process of muscle fibers in this zone. Szent-Györgyi [12] has mentioned the importance of studying muscle in relation to cardiovascular disease. He has pointed out that myosin, if not linked to actin, has a striking affinity for ions, which it binds so strongly that its physical state, charge, and solubility depend on the quality and the quantity of ions present. Since contractility of smooth muscle depends on actomyosin, it becomes important to study ionic changes in this complex as a function of age. It is of interest to note that the changes which comprise the major portion of the aging pattern in the splenic artery are similar to those which Josué [13] and Loeb and co-workers [14] produced in arteries of the rabbit with an injection of epinephrine hydrochloride over four decades ago and which included degeneration of muscle as well as calcification of elastic elements. It is therefore important to determine whether such changes represent a mechanical effect or a chemical one influenced by hormonal activity.

Several important factors, therefore, deserve consideration in understanding the genesis of arteriosclerosis, some apparently mechanical, others metabolic in character. To what extent these factors influence one another and to what extent they are influenced by genetic factors are important problems demanding investigation. According to Burton,[15] the principal mechanical stresses in blood vessels are those produced by hydrostatic tensions, while the constrictive forces are produced by muscular contraction. The elastic elements of the arterial wall may have several related physiological functions, as follows:

1. The internal elastic lamella may limit the dilatation produced by increase of hydrostatic pressure.

2. Elastic elements of the media as in typical elastic arteries probably play a role in the transmission of the rapidly passing pulse waves and produce the rebound from the crest of such a wave.

3. The external elastic lamella may limit the action of smooth muscle in producing contraction of the vessel wall.

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14. Loeb, L., and Githens, T. S.: Am. J. Med. Sc. **130**:658, 1905. Loeb, L., and Fleisher, M. S.: Ibid. **132**:1, 1907.

15. Burton, A. C.: Am. J. Physiol. **164**:319, 1951.

The natural tendency of the internal elastic lamella at rest is to assume the form of a gracefully undulating band. Dilatation of the vessel wall by an increase of hydrostatic pressure straightens the lamella, while constriction serves to accentuate its undulating characteristic. As we have stated, the alternation of these processes eventually produces focal areas of fraying and calcification, which appear to play an important role in plaque formation. This focal characteristic of arteriosclerosis related to functional demand has also been stressed recently by Taylor, Baldwin, and Hass.[16] The elastic elements within the media of arteries are constantly distorted by pulse waves. With the passage of time they fray and calcify; it is interesting that, as in the intima, there appears to be an increase of lipids in the media.[17] On the other hand, the external elastic lamella surrounds the artery with a sheath of longitudinal fibers, which in the resting state do not show as marked undulations as the internal lamella. In a relatively well-preserved state they may limit the amplitude of constrictions of the muscle fibers, but when they calcify they probably also limit vascular dilatation by acting as a splint.

The outer half of the arterial wall deserves special attention in the case of the renal and splenic vessels. Vascular function differs in different anatomic locations according to the function of the end organ which is supplied. An artery supplying the spleen differs from a cerebral artery. It is likely that autotransplants of arteries if they were made early enough in life would show adaptive changes in accordance with the function of the organs which they would supply; such experiments are being planned. The spleen and the kidney represent organs which are sensitive to adrenergic stimuli, and in times of stress the spleen forcibly contracts. Its arteries and arterioles probably react as rapidly as the capsule, and the splenic artery must therefore rapidly adapt to a great increase in peripheral resistance. This probably represents the cause of the relatively rapid muscular degeneration and calcification in the splenic artery. The same is true of the renal artery, but here the calcifying external lamella is capable of affording some protection, and degeneration of muscle is rarely noted. The difference in reactivity of the external lamellae of these two arteries is not apparent, but in both arteries the end effect of calcification of the outer portion of the vessel wall probably represents a protective effect. The support of rigid pancreatic tissue may delay calcification of the external lamella of the pancreatic artery.

These histochemical studies, therefore, indicate a close relation between structure and function of various elements of the arterial wall, on the one hand, and chemical changes leading to the deposition of lipids, on the other. Stresses and strains appear to play an important role in the production of certain degenerative processes as well as in certain adaptive phenomena. In this report these factors as they are related to the splenic and renal arteries and their response to adrenergic stimuli have been stressed. In a subsequent paper we shall deal in greater detail with the physical forces as they relate to the formation and distribution of intimal plaques in large arteries such as the pulmonary artery and the various segments of the aorta.

16. Taylor, C. B.; Baldwin, D., and Hass, G. M.: Localized Arteriosclerotic Lesions Induced in the Aorta of the Juvenile Rabbit by Freezing, Arch. Path. **49**:623 (June) 1950.

17. Buck, R. C., and Rossiter, R. J.: Lipids of Normal and Atherosclerotic Aortas: A Chemical Study, A. M. A. Arch. Path. **51**:224 (Feb.) 1951.

In the splenic artery a distinct correlation of arteriosclerosis and hypertension appears only in the age group 31 to 50 years, where there are six cases of hypertension in the Negro series and four in the white. One-half of the hypertensive Negroes show bone formation along the internal elastic lamella, compared with 23% of the total age group, while one-fourth of the white hypertensive persons show this change, compared with only 9% of the total group. No conclusions are drawn from these figures because of the relatively few cases of hypertension. Calcification of the outer half of the media apparently unrelated to elastic tissue change averages 2.5+ among hypertensive members of both races, compared with an average of approximately 1.4+ for Negroes and 1.8+ for white persons of this age group. In two white diabetic patients in this age group bone formation also is found in this outer medial zone. In all of this study three white persons and one Negro showed this intensity of calcification of the outer media; the remaining two nondiabetic patients had hypertension.

In succeeding age periods, bone formation occurs along the internal elastic lamella with either about the same or lower frequency in hypertensive persons as in the corresponding group as a whole, and the intensity of calcification of the outer half of the media is likewise not significantly different in hypertensive and nonhypertensive persons. There are no diabetic persons more than 50 years of age, and no distinct trend occurs among cancer patients. Tuberculosis does not appear to alter significantly the rate or the intensity of these aging changes.

SUMMARY

Renal and splenic arteries from 61 Negro and 147 white patients have been studied by comparing sections treated with elastic tissue stains and microincinerated preparations. Both splenic and renal arteries show straightening, increasing density, and thickening of the internal elastic lamella with increasing age, but relatively less fraying than has been observed in the coronary artery. Such observations may be correlated with the low incidence of intimal plaque formation and thrombosis in renal and splenic vessels.

The renal artery shows progressive calcification of the external lamella and of elastic elements extending into the media from both lamellae. This process has not been observed in the splenic artery; instead there is progressive calcification of the outer half of the media, which does not appear to be associated with elastic tissue elements and which was found to be most severe in a few cases of hypertension and diabetes.

Renal artery aging appears to be more rapid in Negroes than in white persons, and certain qualitative differences also have been observed between the two races. These may possibly be correlated with the higher incidence of hypertension in Negroes.

Etiological factors possibly responsible for these vascular changes have been noted and discussed, including the role of epinephrine.

CORTISONE AND MATRIX FORMATION IN EXPERIMENTAL SCORBUTUS AND REPAIR THEREFROM

With Contributions to the Pathology of Experimental Scorbutus

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PERUSAL of current medical literature indicates that cortisone has biologic properties affecting numerous diverse mammalian chemical systems and tissues and that it is a therapeutic agent of panacea status, particularly for those diseases assumed to have their origin in collagenous structures. Its proved value in the treatment of rheumatic fever and rheumatoid arthritis, unexplained by known metabolic effects, challenges experimentation by pathologists. A prevailing idea is that cortisone has influence on mesenchymal tissues in relation to the maintenance or integrity of intercellular substances, of which collagen is the chief or an important component.

This study was undertaken to ascertain the influence of cortisone on a specific effect of experimental scurvy in the guinea pig—the cessation of formation of the intercellular substances of fibrous tissue, bone, and cartilage—and on the resumption of formation of these materials in repair after ascorbic acid has been restored to the diet.[1] Some pertinent chemical studies, which will be reported separately, were made by one of us. This report deals only with morphologic observations.

Our experiments were directed to answer these questions: 1. Will cortisone administered after the development of the lesions of scurvy produce a demonstrable histologic effect? 2. Will the daily concurrent administration of cortisone modify the progress of events in guinea pigs fed a diet deficient in ascorbic acid? 3. Will cortisone modify the reparative sequences resulting from ascorbic acid treatment of scorbutic guinea pigs?

These questions were all decisively answered in the negative.

We present details of our experiments, adequate for the purpose of critical appraisal of the results obtained.

From the Division of Nutritional Research, The Children's Hospital.

This study was supported in part by grants from The Nutrition Foundation and the Williams-Waterman Fund for the Combat of Dietary Diseases.

1. Wolbach, S. B.: Controlled Formation of Collagen and Reticulum: A Study of the Source of Intercellular Substances in Recovery from Experimental Scorbutus, Am. J. Path. 9:689, 1933.

EXPERIMENTS

The guinea pigs were studied in three groups.

GROUP 1 (Table 1).—Nineteen animals, ranging in weight from 159 to 190 gm., were restricted to the ascorbic-acid-free diet of Boyle, Bessey, and Howe.[2] Cortisone acetate[3] was injected intraperitoneally in 12-, 12.5-, 15-, and 25-mg. daily doses from the 18th to the 22d day, inclusive, of the experiment into five animals (done in duplicate in all except those with the highest dosage level, corresponding to 60, 61, 82, 85, and 140 mg. per kilogram of body weight, respectively). Six hours after the final injection they were etherized lightly, bled by heart puncture, and killed, and immediate postmortem examination was done. Non-cortisone-treated scorbutic controls were killed under similar conditions on the same day. Five animals were selected for repair; two were given ascorbic acid (50 mg. by mouth daily) without cortisone, and three, the same amount of ascorbic acid supplemented in each instance with a single intraperitoneal injection of cortisone actetate daily, respectively, in amounts of 88, 117, and 137 mg. per kilogram of body weight. One guinea pig without the cortisone treatment and one with this treatment died after 24 hours. The other three were killed after 72 hours on the repair schedule. The guinea pigs given ascorbic acid (25 mg. daily by mouth) were used as diet controls and were killed at the termination of the experiment.

Results.—In the group selected for cortisone treatments clinical evidences of scurvy appeared after an average lapse of 12.2 days, compared with 12.1 days for the untreated group, a result indicative of the strictly comparable categories of the two groups for the experiment. There were two deaths in the group of seven untreated scorbutic animals, on the 23d and 24th days of the diet. However, the whole series gave no indication that the cortisone prolonged the survival time of the guinea pigs with the scorbutic diet. The average weight loss from the maximum weight achieved in grams per day in the untreated was 4.94; in the treated, 5.26 gm. The gross findings in extent and intensity of lesions as indicated by hemorrhage showed no differences in the untreated and the treated.

In the repair group, the two animals which died after 24 hours showed no significant differences in weight loss, viz., 8.4 and 6.0 gm. in the cortisone-treated and the untreated, respectively. The gross findings were similar. In the 72 hour group the cortisone-treated animals continued to lose, while the untreated gained, but in general behavior and postmortem gross findings they were indistinguishable.

GROUP 2 (Table 1).—This group comprised 18 guinea pigs, ranging in weight from 169 to 248 gm. The scorbutic diet employed in this experiment was a commercial rabbit chow[4] previously ground, heated overnight in an electric oven at 100 C., supplemented with dried yeast (40 gm. to every 1,000 gm. of dried food), and then mixed with water to form a coarse paste. Cod liver oil (1 cc. per animal) was given twice weekly as in the schedule recommended by Boyle, Bessey, and Howe.[2] Five animals on this diet were given cortisone acetate daily intraperitoneally at the following levels: two at 2.5 mg. per kilogram of body weight; two at 5 mg./kg., and one at 10 mg./kg. Another five were given cortisone acetate in the same manner at the same dosage levels and distribution as to numbers, but beginning only after the scorbutic syndrome was well established. Two others were selected for ascorbic acid repair (50 mg. per day) as influenced by cortisone and given two and three doses of the acetate, respectively, at the 2.5 mg./kg. level, and killed after 48 and 72 hours. Of the remaining six, three were used

2. Boyle, P. E.; Bessey, O. A., and Howe, P. R.: Rate of Dentin Formation in Incisor Teeth of Guinea Pigs on Normal and on Ascorbic Acid-Deficient Diets, Arch. Path. **30**:90 (July) 1940.

3. The preparation used was "suspension cortone acetate with benzyl alcohol 1.5%," a saline suspension containing 25 mg. of cortisone acetate in 1 cc., made by Merck & Company, Inc., Rahway, N. J.

4. Purina rabbit chow checkers,® made by the Ralston Purina Company, St. Louis.

as scorbutic controls and three as diet controls. The duration of the experiment was from 21 to 30 days, individual animals either dying, being killed in a moribund or severely scorbutic state, or, in the repair and diet control group, killed at the termination of the experiment. The manner of killing was as in Group 1.

Results.—Symptoms in the "prophylactic" cortisone group appeared after an average interval of 17.4 days; in the non-cortisone-treated group, after 16.3 days. In each of the three categories of guinea pigs of this group, five with untreated scurvy, five with scurvy and prophylactic administration of cortisone, and five with scurvy and reparative administration of cortisone, there were three deaths before the termination of the experiment with average survival times, respectively,

TABLE 1.—*Effect of Cortisone on Scurvy*

Category	Animals	Initial Weight, Gm.	Final Weight, Gm.	Adrenal Weights, Mg.	Ratio of Adrenal Wt. to Final Body Wt., Mg./Gm.
Scurvy	4	211	147	203	1.39
(Standard error)		±12.64	±2.45	±14.45	±.0208
Scurvy plus "curative" cortisone	5	181	145	169	1.19
(Standard error)		±3.29	±6.49	±13.31	±.0436
Scurvy plus "prophylactic" cortisone	5	187	140	205.4	1.43
(Standard error)		±5.61	±2.005	±13.42	±.0108
Controls	5	231	285.1	153	0.55
(Standard error)		±9.22	±11.97	±6.05	±.0016

TABLE 2.—*Effect of Cortisone on Repair in Scurvy*

Category	Animals	Initial Weight, Gm.	Final Weight, Gm.	Adrenal Weights, Mg.	Ratio of Adrenal Wt. to Final Body Wt., Mg./Gm.
Scurvy	7	234	173	218	1.28
(Standard error)		±5.38	±6.22	±11.75	±.057
Scurvy + repair—72 hr.	2	209	207	219	1.06
(Standard error)		±22.2	±5.24	±28.8	±.0239
Scurvy + repair + cortisone—72 hr.	4	232	195	162	0.84
(Standard error)		±15.92	±9.2	±5.92	±0.0706
Scurvy + repair—96 hr.	4	209	227	191	0.84
(Standard error)		±4.28	±7.78	±6.87	±.0023
Scurvy + repair + cortisone—96 hr.	4	256	230	183	0.83
(Standard error)		±4.75	±13.55	±9.6	±.022
Controls	5	231	285.1	153	0.55
(Standard error)		±9.32	±11.97	±6.05	±.0016

of 24, 23, and 23.7 days. Average weight losses from the maximum weights achieved were 6.94, 7.15, and 5.87 gm. per day in the same order as above mentioned. The over-all picture as related to postmortem findings, with especial reference to hemorrhage, though differing somewhat in the individual animals in any one group, was essentially the same.

Both the guinea pigs in which repair induced by ascorbic acid was studied during administration of cortisone continued to lose until the last 24 hours, when both gained—the 72-hour animal 7.0 gm. and the 48-hour one 3.7 gm. Again, gross findings were consistent with the duration of repair as ordinarily found without the implementation of cortisone. Adrenal weights will be discussed as related to final body weights after consideration of Group 3.

GROUP 3 (Table 2).—This group included 18 animals, ranging in weight from 240 to 300 gm. Here cortisone acetate was employed purely reparatively, at a level of 5 mg. per kilogram of

body weight. The diet was that used in Group 2, similarly supplemented. Animals were divided into the following categories: (*a*) diet controls, 2 animals; (*b*) straight scurvy, four animals; (*c*) scurvy plus ascorbic acid, 50 mg. per day for three days, killed after 72 hours on repair, two animals; (*d*) scurvy plus ascorbic acid, 50 mg. per day for three days, plus cortisone, two animals; (*e*) scurvy plus ascorbic acid, 50 mg. per day for four days, killed after 96 hours on repair, four animals; (*f*) scurvy plus ascorbic acid, 50 mg. per day for four days, plus cortisone, four animals.

Only animals that were judged to be definitely scorbutic were chosen for repair. Criteria of scurvy were the presence of any three of the following symptoms: consistent loss of weight for two days or longer, diarrhea, bloody stools, tender extremities, assumption of the "face ache" position, inability to use the hind legs. This occurred between the 20th and the 23d day. An attempt was made to match every cortisone-treated guinea pig with another guinea pig of approximately the same weight and of about the same degree of scorbutic symptoms. The manner of killing was as in previous series. Adrenal weights were noted.

Results.—There seemed to be no essential difference between the repair animals with and those without cortisone. Symptomatically the situation was unaltered. In the 72-hour noncortisone group weight gains were 5.7 gm.; in the cortisone group, 10.9 gm. In the 96-hour animals, one animal in each group continued to lose weight—5.2 gm. in the noncortisone guinea pig, compared with 6.4 gm. in the cortisone guinea pig, while those that gained increased by 11.5 and 19.4 gm., respectively. The gross findings at postmortem examination showed the same variation in both cortisone and noncortisone guinea pigs with neither acceleration nor deceleration of repair in the cortisone-implemented group.

COMMENT ON TABULATED RESULTS

Tables 1 and 2 include, of the 55 used for the three groups described, only the guinea pigs (40 in number) on which chemical studies were made and whose adrenals were weighed. All of the 55 were studied histologically. In all instances the preparations included the adrenals, the costochondral junctions, and the proximal tibial and distal femoral epiphyses. We are confident that the animals included in the tables are thoroughly representative of the whole from the standpoint of pathology.

It was not until our experiments were under way that we realized that evidence corroborative of histologic observations was desirable and decided to (1) weigh adrenals accurately and compute the ratios of adrenal weight to body weight; (2) determine mucoprotein values of serum by tyrosine and carbohydrate analyses; (3) determine glucosamine values of serum; (4) determine lipid values of serum. Sera were analyzed for total cholesterol and lipid-phosphorus and the ratio of cholesterol to lipid-phosphorus (expressed as lecithin) computed.

The gross effect of scurvy on the adrenal of the guinea pig and the response to ascorbic acid therapy, as shown by Tables 1 and 2, were predictable on the basis of current knowledge. Longer periods of therapy are probably necessary to restore the adrenal weight/body weight ratio to the normal. For the duration of our experiments the cortisone administered did not modify the gross response of the adrenal to the scorbutic diet or the repair following ascorbic acid therapy.

The chemical studies also failed to give evidence that the cortisone administered had influence on the progress of scurvy. In those animals which received ascorbic acid (Table 2) there was a somewhat greater drop of serum mucoprotein tyrosine levels in those given cortisone as determined after both 72 hours' and 96 hours' repair.

The chemical studies will be reported separately by Dr. Maddock.

HISTOLOGIC STUDIES

Complete coverage of all organs and tissues was made in representative guinea pigs of all three groups. Bones (ribs, including costochondral junctions, and the knee joints for distal femoral and proximal tibial metaphyses) and the adrenals were processed from every guinea pig.

Paraffin embedding was used for soft tissues; celloidin (pyroxylin) embedding after decalcification in 5% aqueous nitric acid was used for bone. Zenker's fixative fluid was routinely used, but neutral 10% formalin and absolute alcohol were used as occasion required. Hematoxylin and eosin, Mallory's methylene blue and eosin, and Mallory's aniline blue, orange G, acid fuchsin connective tissue stains were used, supplemented by scarlet red staining for lipids and Best's stain for glycogen for obvious purposes. Mallory's connective tissue staining method was used in the study of all skeletal structures for verification and extension of observations made on hematoxylin and eosin preparations.

Our studies of soft tissues and organs we regarded as incidental but desirable, and the results will be presented in brief. The bones of the knee joint provided the best material for our purpose. The costochondral junctions of ribs, because decalcification was accomplished in Zenker's fixative without further use of acid and because thin paraffin sections were possible, were useful for following with greatest clarity the histologic results of the deficiency and reparative sequences in matrix deposition of bone and connective tissue.

Epiphysial Cartilage

The cessation of cartilage matrix formation in scurvy and the resumption of matrix formation in repair, and the accompanying cytologic changes, could be followed only in the epiphysial cartilage of the long bones. The present study has provided proof that in scurvy the failure of formation of the matrix of cartilage is as prompt and as complete as that of the matrices of bone and connective tissue, and that in repair the resumption of its formation is likewise as prompt and conspicuous as that of the matrices of bone and connective tissue. The value of the epiphysial cartilage of the long bones for our purposes resides in the rapidity of growth sequences as compared with those of the ribs in young animals and the preservation of relationships not preserved in the costochondral junctions.

In comparing cortisone- and non-cortisone-treated guinea pigs of all three groups in the study of matrices we selected known regions of normal active growth for observations on the failure of matrix deposition and on the resumption of deposition during repair resulting from ascorbic acid administration. Accordingly, for the fibrous tissue sequences, the outer layer of the periosteum, and sites of attachment of ligaments and muscles were studied; for bone matrix, the inner periosteal layer, the endosteal regions of most active normal growth attending remodeling, and the appositional deposit regions on the diaphysial side of the epiphysial cartilage; for cartilage matrix, the epiphysial cartilage zones where normally growth of cartilage cells and matrix deposition are prominent. Cytologic changes resulting from the deficiency and following institution of repair were most prominent in epiphysial cartilages, but their study was hampered by technics necessarily used for making preparations of bone.

For the study of reparative sequences following ascorbic acid therapy with and without cortisone administration, the entire *Gerüstmark* was most useful, and

incidentally we have wholly satisfactory confirmation of Follis' demonstration [5] that the *Gerüstmark* results from the reaction to trauma occasioned by infraction. In 1926, Wolbach and Howe [6] stated that the source of the fibroblast-like cells composing the *Gerüstmark* was osteoblasts and that following orange juice therapy they became the source of the deposition of osteoid matrix and resumed the morphologic aspect of osteoblasts.

The study of reparative sequences with and without cortisone following ascorbic acid administration revealed without exception that the entire *Gerüstmark* is a source of osteoid deposition and that in a period of 72 hours there is produced the trabecular bone characteristic of callus formation in the repair of fractures (Figs. 1 and 2). Corollary to Follis' demonstration is the explanation that the *Gerüstmark* of scurvy is essentially an internal callus, the extent of which is determined by the degree of infraction, and incapable of bone formation until ascorbic acid is restored to the diet. As such we found it valuable for our comparisons when searching for an influence of cortisone on matrix formation.

In our opinion, the least adequately described effect of scurvy in the guinea pig is that on epiphysial cartilage. In 1942, Wolbach and Bessey [7] stated "no satisfactory experimental study of the effects of ascorbic acid deficiency upon cartilage has been done in spite of the opportunities offered in epiphyseal cartilage growth." Follis, Park, and Jackson [8] stated that "cells of the epiphyseal and costal cartilages continue to proliferate and mature" with reference to human scorbutus and in confirmation of an earlier opinion expressed by Park, Guild, Jackson, and Bone.[9]

The present study has revealed that in young guinea pigs the matrix formation of epiphysial cartilage cells ceases as a result of ascorbic acid deficiency just as promptly and conspicuously as does the matrix formation of bone and connective tissue cells and that the cartilage cells do not mature until ascorbic acid is given.

In well-developed scurvy, such as is found in 18 to 24 days [10] in guinea pigs restricted to an ascorbic-acid-free diet, the deficiency of cartilage matrix is very apparent and is accompanied by prominent changes in the cartilage cells and great distortions of cartilage cell columns, all of which are present before the occurrence of infraction or the formation of a *Gerüstmark*. All zones of the epiphysial cartilage in which multiplication and growth of cartilage cells occur exhibit the deficiency effects, so that the abnormal cartilage is bounded on the epiphysial side by the layer

5. Follis, R. H. Jr.: Effect of Mechanical Force on the Skeletal Lesions in Acute Scurvy in Guinea Pigs, Arch. Path. **35**:579 (April) 1943.

6. Wolbach, S. B., and Howe, P. R.: Intercellular Substances in Experimental Scorbutus, Arch. Path. & Lab. Med. **1**:1 (Jan.) 1926.

7. Wolbach, S. B., and Bessey, O. A.: Tissue Changes in Vitamin Deficiencies, Physiol. Rev. **22**:233, 1942.

8. Follis, R. H.; Park, E. A., and Jackson, D.: The Prevalence of Scurvy at Autopsy During the First Two Years of Age, Bull. Johns Hopkins Hosp. **87**:569, 1950.

9. Park, E. A.; Guild, H. A.; Jackson, D., and Bone, M.: The Recognition of Scurvy with Especial Reference to the Early X-Ray Changes, Arch. Dis. Childhood **10**:265, 1935.

10. Because the pathologic aspect of experimental scurvy is the result of retardation of the deposition of intercellular substances which accompanies growth, the degree or severity of the lesions is influenced by the general nutritional state of the animal and is expressive of the growth which occurred while the animal was fed the deficient diet. We have kept this in mind in matching animals for comparative purposes, because we have found, in general, that those guinea pigs which best maintained their weight during the experiment have shown the most conspicuous lesions.

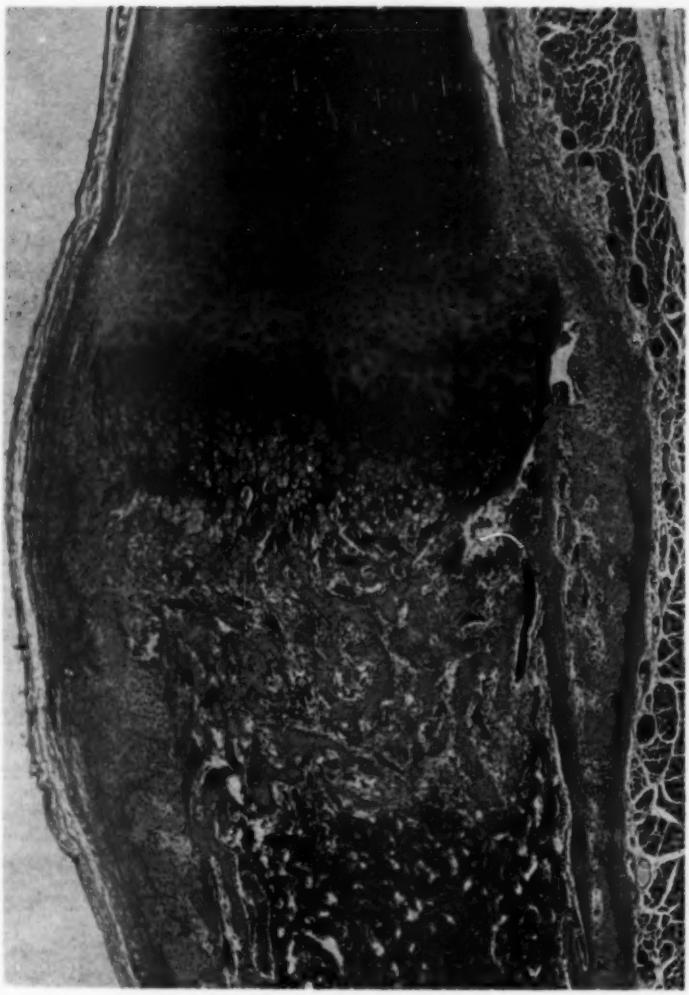

Fig. 1.—Costochondral junction of a guinea pig that had been restricted to an ascorbic-acid-deficient diet for 24 days and then allowed 96 hours' repair. Note the external calluses, the internal callus, which before repair was a typical *Gerüstmark*, and the persistent infraction. The repair of the growth cartilage, as explained in the text, is less evident than that in the epiphysial cartilage of long bones. Hematoxylin and eosin stain; × 35.

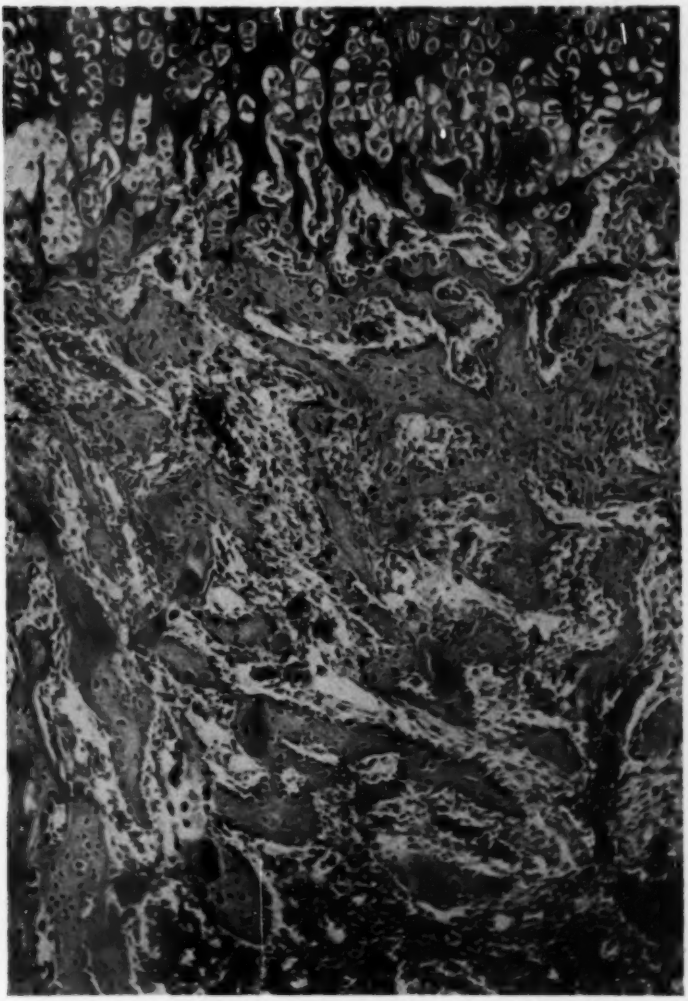

Fig. 2.—Detail of Figure 1. Hematoxylin and eosin stain; × 130.

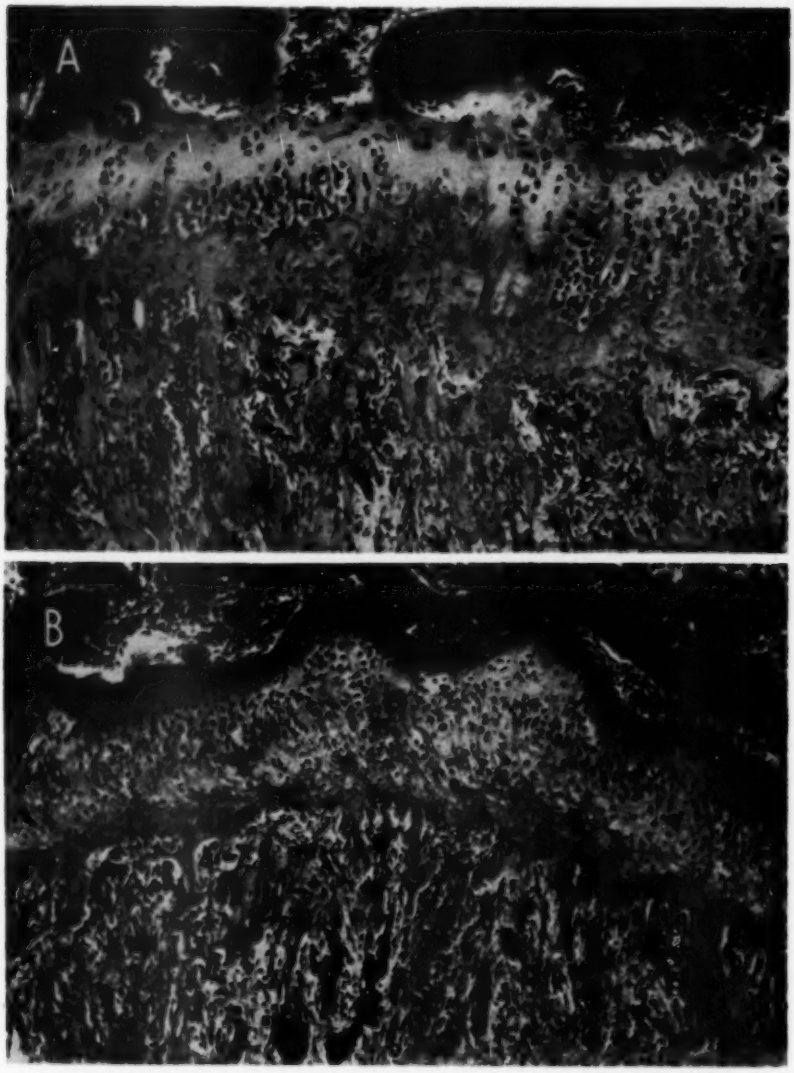

Fig. 3.—*A*, proximal epiphysial cartilage of the tibia of a guinea pig showing late scurvy. The animal had been restricted to an ascorbic-acid-free diet for 30 days. Note the disappearance of matrix and the disorientation of the columns in a zone bounded above (epiphysial side) by the "reserve cartilage" and below (diaphysial side) by calcified cartilage which had formed before the establishment of the scorbutic state. Hematoxylin and eosin stain; × 125.

B, proximal epiphysial cartilage of the tibia of a guinea pig showing less advanced scurvy. This animal had been fed an ascorbic-acid-free diet with concurrent daily doses of cortisone acetate, 5 mg. per kilogram of body weight, for 24 days. Mallory's connective tissue stain; × 125.

of reserve or indifferent cartilage (Zone 1 of Dodds and Cameron [11]) and on the diaphysial side by a zone of calcified cartilage matrix deposited before the deficiency developed (Zone 5 of Dodds and Cameron [11]) (Fig. 3).

The degree of change in the cytologic aspect of the cartilage cells and the deficiency of matrix are not uniform across the epiphysial disk but vary in locations for which no explanation was found. In advanced (presumably absolute) scurvy there is in some locations complete absence of matrix, as demonstrated by hematoxylin and eosin and connective tissue stains. In such locations the cartilage cells are small, with compact cytoplasm, distorted in shape, often flattened in a plane

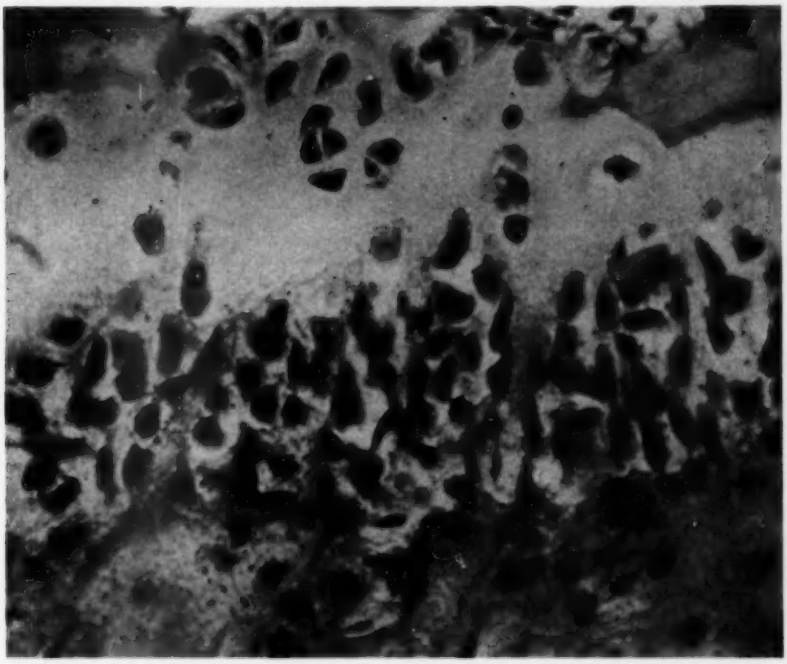

Fig. 4.—High power detail of Figure 3. Hematoxylin and eosin stain; × 550.

transverse to the long axis of the bone, sometimes crescentic in shape, with the concavity on the epiphysial side (Fig. 4). At earlier periods there are less striking changes, but in all cases there is not much increase in size of the cell and the vacuolated cytoplasm and peripheral striations of cartilage cells approaching maturation are absent.

The response to ascorbic acid therapy is evident in 24 hours, both in the resumption of growth of cartilage cells and in the matrix deposition. In 48 hours, enough

11. Dodds, G. S., and Cameron, H. C.: Studies in Experimental Rickets in Rats: I. Structural Modifications of the Epiphyseal Cartilages in the Tibia and Other Bones, Am. J. Anat. **55**:135, 1934.

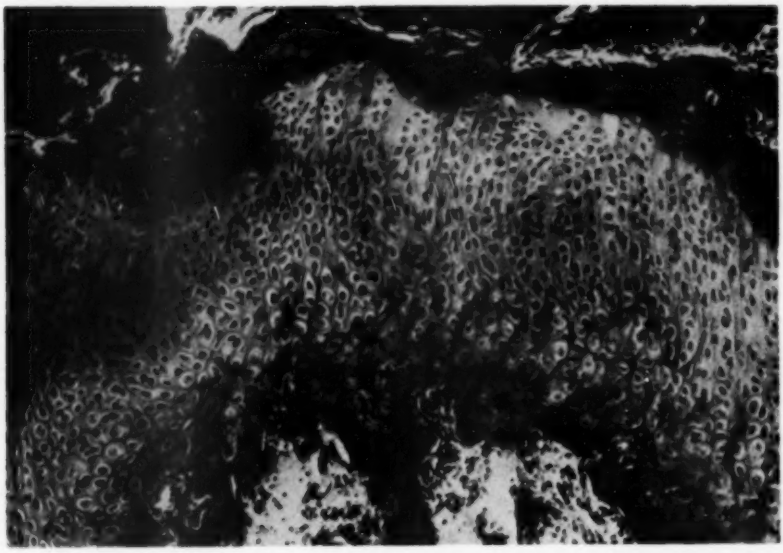

Fig. 5.—Distal epiphysis of the femur of a guinea pig showing repair of scurvy. The animal had been restricted to an ascorbic-acid-deficient diet for 24 days, after which ascorbic acid had been administered for 4 days. A region of infraction is shown. Note the approximately normal growth cartilage and the persistence of the calcified cartilage zone on the diaphysial side. Mallory's connective tissue stain; × 125.

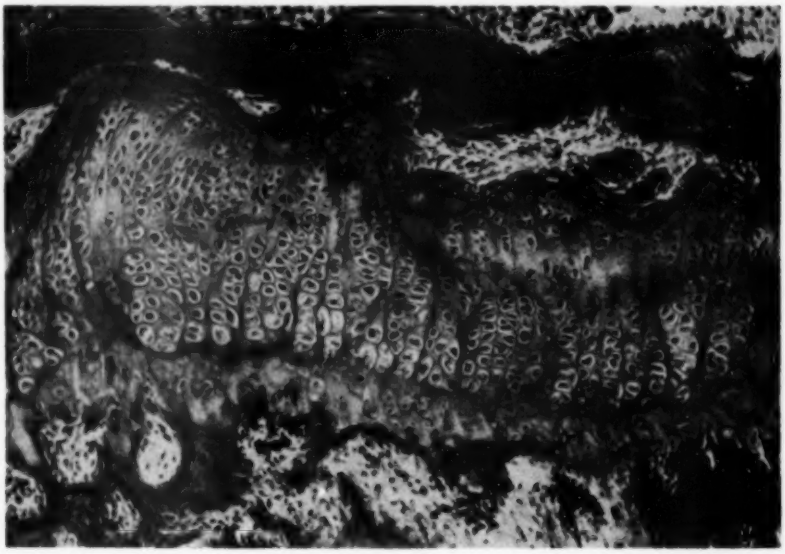

Fig. 6.—Guinea pig repair of scurvy following administration of ascorbic acid and cortisone. For 24 days the animal had been fed an ascorbic-acid-free diet. It then was treated with ascorbic acid and cortisone (5 mg. of cortisone acetate per kilogram of body weight), administered daily for 4 days. The growth cartilage has approached normal, but the calcified cartilage in the region selected has not been penetrated by blood vessels. While there are differences in details of Figures 5 and 6, comparisons of all of the guinea pigs listed in Table 2 have shown no quantitative or qualitative differences in the guinea pigs which received cortisone during the reparative period.

matrix is deposited to produce in some regions the effect of capsules, and enlarged, maturing cartilage cells have become finely vacuolated. In 72 hours, cartilage cells are found which appear fully mature,[12] and the columnar arrangement is restored. In 96 hours, except for the zone of calcified cartilage matrix on the diaphysial side, appearances approach normal, and some penetration of diaphysial blood vessels may be present. All of these reparative events, uninfluenced by cortisone administration, take place in the epiphysial cartilage even where this is separated from the diaphysis by zones of infraction (Figs. 5 and 6).

The reparative sequences shown by deposition of collagen in fibrous tissue structures and of osteoid in callus formation, by resumption of appositional bone growth, and by restoration of the cytologic aspects of fibroblasts and osteoblasts are too well known to need description here. It should be mentioned, however, that the whole of the *Gerüstmark* rapidly becomes a region of osteoid deposition, visible after 24 hours in the ribs, which can be sectioned without special treatment after being fixed in Zenker's fluid. In 96 hours the *Gerüstmark* in ribs and long bones becomes as fully occupied by unoriented trabeculae of bone as is any early bone callus, however induced.

The account we have given of the distinctive lesions of experimental scurvy entailing some new observations on cartilage and the *Gerüstmark* is presented mainly to make known the criteria which we utilized for the comparison of cortisone-treated and non-cortisone-treated scorbutic guinea pigs of the three groups employed.

In no detail have we been able to demonstrate any influence of cortisone as administered by us on the progress of scurvy in the guinea pig or on the deposition of matrices in repair.

Other Tissues and Organs

Adrenal Glands.—A histologic study of the adrenals of all of the 55 guinea pigs used in the three groups was made. The failure of cortisone to affect the adrenal weight/body weight ratio either in scurvy or in the repair induced by ascorbic acid is shown in Tables 1 and 2. The enlargement of the adrenal in experimental scurvy is usually referred to as hypertrophy and in general has been regarded as distinctive of ascorbic acid deficiency. In recent years the diminished lipid content of the cortex has been regarded by some authors as evidence that ascorbic acid plays a specific role in the synthesis of the cortical lipids. It is necessary to point out that we can find no histological differences between the enlarged adrenal glands of experimental scurvy and those enlarged as a result of two other conditions, namely, excessive administration of vitamin A[13] and, as shown by Blumenthal and Loeb,[14] underfeeding. In all three conditions there is an extraordinary increase of mitoses throughout the fascicular zone. Mitosis often seems arrested at the metaphase, as frequently cells are seen with dispersed and fragmented chromosomes.

12. Maturing and matured cartilage cells are often referred to as hypertrophic, a term which is inappropriate for cells in any stage of normal growth.

13. Wolbach, S. B.: Vitamin A: Deficiency and Excess in Relation to Skeletal Growth (Ludvig Hektoen Lecture), Proc. Inst. Med. Chicago **16**:118 (April 15) 1946; J. Bone & Joint Surg. **29**:171, 1947.

14. Blumenthal, H. T., and Loeb, L.: Two Antagonistic Effects of Underfeeding on Adrenal Cortex of Guinea Pig, Am. J. Path. **18**:615, 1942.

The cells of the fascicular zone are swollen, pale, finely vacuolated, and contain but little lipid. The glomerular zone becomes atrophic, the cells small and without lipid droplets.

In all three conditions the changes described are reversible. The diet used by Blumenthal and Loeb [14] was their normal diet and included greens. In unpublished experiments made with the late P. R. Howe, the effects of vitamin A excess were not prevented by administering 250 mg. of ascorbic acid daily to the very young guinea pigs used (weighing less than 200 gm.).

Our histologic studies of the adrenals failed to bring to light any change of response to cortisone whether this was given prophylactically, or after the establishment of scurvy. Cortisone did not modify the effects of ascorbic acid therapy on the adrenals as followed by us up to 96 hours, at which time, in spite of the still somewhat increased adrenal weight/body weight ratio, the return to normal was complete except for a somewhat diminished stainable lipid content.

Lymphoid Tissues.—Spleen, thymus, and lymph nodes were studied, five from straight-scorbutic guinea pigs, six from cortisone-treated scorbutic guinea pigs, and six from guinea pigs which had received prophylactic cortisone treatment. No attempt at quantification was made. We record only the estimations made by comparing sections from cortisone-treated animals with those from normal controls. Four of the five guinea pigs with severe skeletal lesions of scurvy showed essentially normal lymphoid tissues, and one showed marked depletion of lymph nodes, thymus, and spleen. With one exception, the animals treated with cortisone showed moderate to severe lymphoid depletion of spleen, thymus, and lymph nodes. We have no explanation for the inconsistencies found in this small series. Our impression is that the lymphoid tissues of the guinea pig are not so responsive to cortisone as those of the rat as reported in the literature.

Skeletal Muscle.—The atrophy of the skeletal muscle in scurvy was not modified in the cortisone-treated guinea pig, nor was the 96-hour repair following ascorbic acid administration.

Thyroid and Parathyroid Glands.—These showed no response to cortisone in any of the three groups of animals.

Pituitary Gland.—This organ was studied in serial sections from five scorbutic guinea pigs, all of which had advanced lesions of bones and had received, on each of four days before they were killed, doses of cortisone acetate as follows: four, 60 mg./kg.; one, 80 mg./kg., and one, 140 mg./kg. Comparison with normal controls and two untreated animals with advanced scorbutic changes revealed no differences.

Pancreas.—Studies of the islets of Langerhans of guinea pigs of all three experimental groups revealed no differences resulting from administration of cortisone.

Liver.—The loss of stainable fat as determined by study of a few guinea pigs was not influenced by cortisone, nor was the increase of fat which followed ascorbic acid therapy. Glycogen studies (Best's carmine after absolute alcohol fixation) were made on 12 livers from guinea pigs as follows: 5 from cortisone-treated, 2 from untreated, 2 from control guinea pigs, 2 from animals treated with ascorbic acid plus cortisone, and 1 showing repair following ascorbic acid administration.

From this small series we could get no indication that cortisone influenced either the depletion of glycogen in scurvy or the restoration of glycogen following ascorbic acid therapy.

Lungs.—We saw no indication that administration of cortisone influenced the incidence or the severity of lung infections at a time when these were prevalent during the experiments.

COMMENT

Our failure to detect an influence of cortisone on the adrenals in scurvy and in repair therefrom as shown by adrenal weight/body weight ratios and histologic observations was contrary to expectations derived from the literature. We do not feel competent to discuss the possible factors involved in the production of the histologic and cytologic defects common to the three conditions: ascorbic acid deficiency, acute inanition, and hypervitaminosis A. Without knowledge of possible differences in the lipids demonstrated by scarlet red, it is rash to suggest that further exploration of the adrenals in these three conditions might contribute evidence that ascorbic acid is related to lipid synthesis and to vitamin A physiology.

We are pleased to record the ease with which the cessation of cartilage matrix formation in ascorbic acid deficiency can be shown in epiphysial cartilages of the long bones and the opportunity afforded by reparative sequences following replacement therapy for the study of the formation of an intercellular material that is poor in collagen by histochemical and microchemical technics.

The explanation of the nature of the *Gerüstmark* suggested by Follis' experiments [3] is offered with conviction of its correctness and with some chagrin because, in addition to the material on which this report is based, we have had for many years equally good evidence from unpublished studies of reparative sequences in experimental scurvy, which one of us made, but which needed the light thrown by Follis.

We are aware, in spite of the clean-cut information afforded by our experiments, that generalizations concerning results observed on humans and animals other than guinea pigs affecting the healing of wounds would not be warranted. Species differences may be important, and there are other factors concerned in wound healing than the formation of intercellular substances. Experimental studies have been few and their results discordant. Retardation of wound healing ascribed to depression of activity of connective tissue was reported in 1950 for the rabbit following large doses of cortisone.[15] Also in 1950, from the same group of authors,[16] another paper appeared, reporting delayed granulation tissue formation in the rabbit and also delayed healing of fractures. Inasmuch as identical illustrations appeared in these two papers, it can be inferred that the same rabbits, at least in part, were reported on. The doses of cortisone acetate varied from 2 mg. to 12.5 mg. per kilogram of body weight. The larger doses were reported as more effective in delaying repair. Spain and associates [17] in 1950 reported similar results in white

15. Plotz, C. M.; Howes, E. L.; Blunt, J. W.; Meyer, K., and Ragan, C.: Action of Cortisone on Mesenchymal Tissues, Arch. Dermat. & Syph. **61**:919 (June) 1950.

16. Howes, E. L.; Plotz, C. M.; Blunt, J. W., and Ragan, C.: Retardation of Wound Healing by Cortisone, Surgery **28**:177, 1950.

17. Spain, D. M.; Molomut, N., and Valhalla, A.: The Effect of Cortisone on the Formation of Granulation Tissue in Mice, Am. J. Path. **26**:710, 1950.

mice in experiments of short duration (one to five days). The dose was 1 mg., given twice daily to mice of 20 to 25 gm. weight (roughly 80 mg./kg. daily for a 25-gm. mouse).

Cortisone (also 17-hydroxycorticosterone—Compound F) ("25 to 100 μg. in 0.1 cc. of 25% alcohol . . . equivalent to 1 mg. of cortisone per cubic centimeter") applied to the skin of rats over long periods has been reported as producing atrophy of the epidermis and its appendages and reduction of the thickness of the dermis "apparently due to loss of substance from the collagenous fibers, the elastic fibers remaining numerous in spite of treatment. Fibroblasts and other cells of the dermal connective tissue were fewer in number."[18]

Shaffenburg, Masson, and Corcoran [19] in 1950 were the first to employ scorbutic guinea pigs for a study of the effects of cortisone administration. They reported that "scurvy caused a marked adrenal hypertrophy which was completely prevented by cortisone but only partially suppressed by DCA" (desoxycorticosterone acetate), also that "cortisone inhibits many of the manifestations of scurvy in the guinea pig while desoxycorticosterone aggravates the condition." Their guinea pigs weighed 280 to 300 gm. Six were used for cortisone studies, the daily doses were 5 mg. of the acetate for 10 days (approximately 16.5 mg./kg.), 7.5 mg. from the 11th to the 18th day (approximately 25 mg./kg.) and 10 mg. from the 19th to the 21st day (approximately 33.3 mg./kg.), at which time the experiment was ended. No histologic studies were made. Their conclusions were drawn from clinical signs and gross findings at postmortem examinations.

Hyman, Ragan and Turner [20] found that the effects of cortisone and corticotropin (ACTH) on scorbutic guinea pigs "were quite similar" and that both agents "ameliorate the manifestations of scurvy in a parallel fashion by a mechanism as yet unknown." Their daily dose of cortisone acetate was 12.5 mg. for guinea pigs of 300 to 400 gm. weight. They noted that in scurvy the greatest enlargement of the adrenal glands occurred in the corticotropin-treated animals, the least in the cortisone-treated. The only histologic studies were made on the adrenal glands. The favorable effect of corticotropin in amelioration of scurvy was regarded as evidence "that cortisone-like steroids can be produced by the scorbutic guinea pig despite absence of measurable adrenal ascorbic acid."

Upton and Coon,[21] in carefully conducted experiments controlled by histologic studies, obtained "no conclusive evidence that either ACTH or cortisone influenced the rate of growth, nutrition or hemorrhagic tendency of the scorbutic animals. . . ." Also, they found that "cortisone and ACTH in both scorbutic and non-scorbutic guinea pigs were without apparent influence upon wound repair." They considered

18. Castor, C. W., and Baker, B. L.: The Local Action of Adrenocortical Steroids on Epidermis and Connective Tissue of the Skin, Endocrinology **47**:234, 1950.

19. Shaffenburg, C.; Masson, G. M. C., and Corcoran, A. C.: Interrelationship of Desoxycorticosterone, Cortisone and Vitamin C in the Genesis of Mesenchymal Lesions, Proc. Soc. Exper. Biol. & Med. **74**:358, 1950.

20. Hyman, G. A.; Ragan, C., and Turner, J. C.: Effect of Cortisone and Adrenocorticotrophic Hormone (ACTH) on Experimental Scurvy in the Guinea Pig, Proc. Soc. Exper. Biol. & Med. **75**:470, 1950. The Effect of Cortisone and ACTH on Experimental Scurvy in the Guinea Pig, Tr. New York Acad. Sc. **13**:167, 1951.

21. Upton, A. C., and Coon, W. W.: Effects of Cortisone and Adrenocorticotrophic Hormone on Wound Healing in Normal and Scorbutic Guinea Pigs, Proc. Soc. Exper. Biol. & Med. **77**:153, 1951.

the possibility that the "connective tissue of the guinea pig may respond to adrenal hormone, if at all, less readily than that of other species studied." They noted elevation of adrenal weight/body weight ratio both in normal and in scorbutic guinea pigs following administration of corticotropin. With cortisone treatment the adrenal weight/body weight ratios were reduced both in normal and in scorbutic guinea pigs. They obtained evidence in the adrenal response to corticotropin in scorbutic guinea pigs, "cortical hyperplasia and sudanophilia," that ascorbic acid might not be essential for the secretion of adrenal hormone.

On the whole, the reports of experimental work with cortisone on wound healing and on the effects of ascorbic acid deficiency have been too few and of a character not favorable for critical appraisal of properties commonly attributed to cortisone in relation to mesenchymal tissues. Our own work clearly shows that administration of cortisone will not prevent in mesenchymal cells the loss of function concerned in formation of intercellular substances caused by ascorbic acid deficiency, nor does it retard or accelerate the formation of intercellular substances in connective tissue, cartilage, and bone during repair of scurvy produced by ascorbic acid therapy.

SUMMARY AND CONCLUSIONS

The epiphysial cartilage of long bones of guinea pigs when observed during active growth is a favorable tissue for the study of the effect of ascorbic acid deficiency on matrix formation and for the study of the sequences of matrix formation in repair after therapy. The *Gerüstmark* of long bones in scorbutic guinea pigs is an internal callus, the result of infraction, but it is unable to form bone matrix until ascorbic acid is restored to the diet.

Cortisone administered in prophylaxis or therapeutically after the development of scurvy does not modify the progress of events discernible by gross and histologic study caused by ascorbic acid deficiency.

Cortisone administration neither accelerates nor depresses the reparative processes in scurvy in the guinea pig. The resumption of formation of intercellular materials is not affected in either direction as far as can be observed by histologic criteria.

Our observations on the adrenals suggest that their response to ascorbic acid deficiency is not peculiar to this condition, nor in our experiments is it materially modified by cortisone administered in the scorbutic state or during repair.

The histologic preparations were made by Mr. John J. Burke, departmental technician.

ROLE OF THE ARACHNOID GRANULATION IN THE DEVELOPMENT OF MENINGIOMA

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THE HISTOLOGICAL similarity of the cells of the endothelial type of meningioma and normal arachnoid cell clusters was first noted by Cleland [1] in 1864 and suggested to him an arachnoid villus derivation of that type of meningioma. Subsequent observers, including Robin,[2] Schmidt,[3] Cushing and Weed,[4] and Mallory,[5] independently reached the same conclusions from noticing this similarity.

These observations, however, apply only to the endothelial type of tumor. Many other types of meningioma have been described according to the predominant cell. The fibroblast is the other main constituent of meningioma, and a great amount of controversy has centered on its origin. Apart from these two main components, others exist, the origin of which is even more obscure. If it is accepted that one type of meningioma derives from a constituent cell of the arachnoid villus, the question is raised whether the others may be similarly derived. To answer this question and to assess the relationship of the arachnoid granulation and the meningioma, it is necessary to analyze the structure of the arachnoid villus to see if its various components can be recognized in the histological types of meningioma. It is also necessary to investigate the anatomical distribution of the arachnoid granulations and correlate this with the common sites of origin of meningioma, as well as analyzing a large series of these tumors to ascertain whether arachnoid granulations have been found in every location where these tumors have been reported.

It is the purpose of this paper to attempt these investigations and to evaluate the evidence for the relationships. This will be done under three headings—Structure, Distribution, and Derivation.

STRUCTURE

Arachnoid Granulations, or Villi.—The villi have been described by Cushing, Weed, and Wegefarth.[6] They consist of small fleshy-looking elevations, often

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1. Cleland, J.: Glasgow M. J. **11**:148, 1864.
2. Robin, C.: J. Anat. **6**:239, 1869.
3. Schmidt, M.: Arch. path. Anat. **170**:429, 1902.
4. Cushing, H., and Weed, L. H.: Bull. Johns Hopkins Hosp. **26**:367, 1915.
5. Mallory, F. B.: J. M. Res. **41**:349, 1920.
6. Cushing, H.; Weed, L. H., and Wegefarth, P.: J. M. Res. **31** (n.s. **26**):1, 1914.

occurring in clusters, appearing as diverticula of the subarachnoid cavity penetrating into the interstices of the dura or into the lumen of the latter's contained blood sinuses. Each is made up of stalk and cap. The stalk consists of a voluminous, delicate, lace-like sleeve, through the axis of which the subarachnoid space is continued, or a small vein passes on its way to the dural sinus. A reticulum of fine fibrous tissue forms the main component of the stalk. This is dense at the periphery but very loose in the center, where it has the staining qualities of myxomatous tissue.

Capping the stalk on all sides is a mesothelial covering of flattened arachnoid cells, with large oval nuclei and lightly staining cytoplasm. In some villi the cap cells form a thin layer, but in certain situations, such as that near the basal cisterns, the greater part of the tuft is composed of these cells. In addition to these villi, arachnoid cell nests occur in almost every part of the dura. These are similar to the cap cells of the villus but form a cluster without a stalk.

The arachnoid granulations are seen as early as 18 months but they increase in size and number with age. The large, hypertrophic villus seen in the adult is known as the Pacchionian body. In older people calcareous deposits are frequently found in these bodies, and they may become ossified.

Histologic Aspects of Meningioma.—Various classification of the meningiomas have been made according to their histological structure, amongst the best being those by Bailey and Bucy[7] (1931), Globus[8] (1937), Cushing and Eisenhardt[9] (1938), del Rio Hortega[10] (1941), and Courville[11] (1945). If an attempt is made to combine these various classifications, three main types, with equivalents in each classification, may be recognized, with several other, less common varieties, differing in number according to the classifier.

The three main types are the following:

1. Endothelial type (meningotheliomatous type of Bailey and Bucy; leptomeningioma of Globus; meningothelial type I of Cushing and Eisenhardt; *exotelioma difuso* of del Rio Hortega; syncytial type of Courville). This is composed of sheets of polygonal cells with indistinct cell boundaries and without an intercellular stroma of either reticulin or collagen. The cells are arranged in nests or alveoli of varying size. Of 263 meningiomas examined in this department, 40% were endothelial.

2. Mixed type (psammomatous type of Bailey and Bucy; "primitive meningioma" of Globus; meningothelial type II of Cushing and Eisenhardt; *exotelioma nodular y lobulado* of del Rio Hortega; transitional or mixed type of Courville). This consists of endothelial cells, with a tendency to be arranged in whorls, and spindle-shaped cells. Some or many of the whorls may become calcified into psammoma bodies. Often a small blood vessel may be seen at the center

7. Bailey, P., and Bucy, P. C.: Am. J. Cancer **15**:15, 1931.

8. Globus, J. H.: Meningiomas: Origin, Divergence in Structure and Relationship to Contiguous Tissues in Light of Phylogenesis and Ontogenesis of the Meninges, with Suggestion of a Simplified Classification of Meningeal Neoplasms, Arch. Neurol. & Psychiat. **38**:667 (Oct.) 1937.

9. Cushing, H., and Eisenhardt, L.: Meningiomas, Springfield, Ill., Charles C Thomas, Publisher, 1938.

10. del Rio Hortega, P.: Arch. argent. de Neurol **24**:7, 1941.

11. Courville, C. B.: Pathology of the Central Nervous System, ed. 2, Pacific Press Publishing Association, Mountain View, Calif. 1945.

of the whorl, and deposits of calcium may appear in its wall as an early stage in the formation of a psammoma body. This type formed 17% of the 263 specimens studied.

3. Fibroblastic type (Bailey and Bucy; Cushing and Eisenhardt; dural fibroblastoma of Globus; *exotelioma laminar* of del Rio Hortega; fibrous type of Courville). This is composed of bundles of spindle cells, which may be arranged in loose whorls, with intervening collagen. In the series studied, 32% were fibroblastic.

Other, less common, types are these:

1. Angioblastic type. Although most pathologists agree on the existence of this type, some, including del Rio Hortega, separate the blood-vessel tumors from the meningiomas altogether. It consists of numerous blood spaces of capillary structure separated by plump polygonal cells, often of foamy appearance, with fine reticulin fibrils forming a network throughout the tumor. These tumors resembled hemangioblastoma of the cerebellum, and Cushing and Bailey [12] noted their predilection for the tentorium and peritorcular region. It is usual to separate the cerebellar tumors from the supratentorial growths, but whether they are regarded as angioblastic tumors of the meninges rather than meningioma depends on individual preference. As the normal arachnoid granulation may contain blood vessels, there is no reason why some of these tumors could not arise from this element, although as blood vessels are so common in the meninges, there are many other possible sites of origin. There were five tumors of this type in the series studied.

2. Osteoblastic type. Any of the three major types of meningioma may contain areas of bone, which may be in relation to calcified psammoma bodies but are usually independent. Only 4 meningiomas in the series of 263 were ossified.

3. Chondroblastic type. These tumors are so rare that Bailey and Bucy do not mention them as one of their nine types, and Cushing and Eisenhardt [9] had only one in their series of 313 tumors. This proved to be a chondrosarcoma. Cushing and Eisenhardt found seven in the literature, one of which (Wolf and Echlin [13]) also was malignant. For all practical purposes this group is unimportant, and the occasional tumor which occurs could be called a chondroma or a chondrosarcoma of the meninges.

4. Melanoblastic type. As melanin is normally present in pial cells over the ventral aspect of the medulla oblongata and the upper part of the cervical cord, it is not surprising that pigmented tumors occur, particularly in the posterior fossa and the spinal cord. These tumors could be classified as melanomas rather than meningiomas, provided other primary sources have been excluded. This is particularly difficult with such a growth as the malignant melanoma. Occasionally fibroblastic or mixed types of meningioma have melanin granules in the cyptoplasm of the tumor cells. These appear to be true melanoblastic meningiomas. Bloch [14] showed that the melanin-bearing cells of the leptomeninx are dopa-negative and therefore are melanophores rather than melanoblasts. As a result, he maintained that they are mesodermal in origin, although Weidenreich [15] believed they are derived from the neural crest. A similar controversy has occurred over the origin of the arachnoid cell. As these cells take up particulate matter, e. g., blood pigment, they may also take up melanin pigment, offering a possible explanation of this type of tumor. Other sources, however, may be responsible for these pigmented tumors of the meninges. Thus, Masson [16] postulated their origin from terminal sense organs, while in a recent case of an unusual pigmented growth occurring in the region of the anterior fontanel of a baby, reported by Clarke and Parsons,[17] an embryological tumor of the retinal anlage was suggested.

12. Cushing, H., and Bailey, P.: Tumors Arising from the Blood-Vessels of the Brain, Springfield, Ill., Charles C Thomas, Publisher, 1928.

13. Wolf, A., and Echlin, F.: Bull. Neurol. Inst. New York **5**:515, 1936.

14. Bloch, B.: Das Pigment, in Handbuch der Haut- und Geschlechtskrankheiten, Vol. I, Berlin, Springer-Verlag, 1927, pp. 434-541.

15. Weidenreich, F.: Ztschr. Morphol. u. Antropol., Sonderheft II, Festschriften für Retzius, 1912, pp. 59-140.

16. Masson, P.: Ann. anat. path. **3**:417 and 657, 1926.

17. Clarke, B. E., and Parsons, H.: Cancer **4**:78, 1951.

5. Lipomatous type. Both endothelial and angioblastic tumors may contain intracellular lipoid material. This may represent a storage or a degenerative process. In support of a storage process is the fact that the nuclei of the cells are intact, and it is known that endothelial cells have a filtering action. Frequently meningiomas with vacuolated cells resembling fat cells in paraffin sections are fat-negative with the Sudan IV stain on frozen sections suggesting some form of degeneration.

6. Sarcomatous type. The potentiality of becoming malignant does not seem restricted to any one type of meningioma, and malignant tumors of each of the types above have been described. Sometimes the cell type changes when the tumor becomes malignant. Only occasionally is the tumor primarily sarcomatous. In the series of 263 meningiomas, 22 (8%) were classified as malignant.

DISTRIBUTION

Arachnoid Granulations.—Quincke [18] noted Pacchionian granulations along the superior longitudinal sinus, the transverse sinus, and the cavernous sinus. Key and Retzius [19] confirmed this, arranging them in this order according to frequency, adding less common sites along the superior petrosal sinus and the middle meningeal veins. Aoyagi and Kyuno,[20] with extreme thoroughness, investigated the whole of the meninges and found arachnoid cell clusters in several additional places. These included the arachnoid (*a*) around the 3d, 4th, 5th, and 6th cranial nerves in their intracranial course, (*b*) at the points where the 3d, 7th, 9th, 10th, 11th, and 12th cranial nerves penetrate the dura, (*c*) around the cervical nerve roots, (*d*) around the hypophysis, (*e*) near the sella turcica, and (*f*) in the basilar plexuses. Le Gros Clark [21] described an enlarged arachnoid granulation projecting upward into the floor of the straight sinus where this is joined by the vein of Galen. Its base was attached to the superior vermis of the cerebellum. Thus the distribution of the granulations is much more widespread than was originally thought as normal components of the meninges.

That this does not exhaust all sites of occurrence became clear when these structures were searched for and recognized in routine autopsy material. They have been seen around the optic nerve in its arachnoid sheath, around the pituitary gland, and in the choroid plexus. The last site is of particular importance, as it has always been difficult to understand how intraventricular meningioma can arise. Those around the pituitary gland offer an answer to the questions about the origin of intrasellar meningiomas, raised by Kirschbaum.[22]

The first 60 arachnoid granulations seen on microscopical examination of routine autopsy material, irrespective of the cause of death, are listed in Table 1.

The granulations encountered around the pituitary gland far outnumbered those at any other site, owing to this being the only tissue examined routinely in all autopsies, the other parts of the meninges and the brain being examined if the history or the macroscopic appearance suggested disease.

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18. Quincke, H.: Zur Physiologie der Cerebrospinalflüssigkeit, Arch. Anat. u. Physiol. u. wissensch. Med. (Du Bois Reymond), 1872, pp. 153-177.

19. Key, A., and Retzius, G.: Studien in der Anatomie des Nervensystems und des Bindegewebes, Stockholm, P. A. Norstedt & Söner, 1875.

20. Aoyagi, T., and Kyuno, K.: Neurologia **11**:1, 1912.

21. Clark, W. E. Le Gros: Brit. M. J. **1**:476, 1940.

22. Kirschbaum, W. R.: J. Neuropath. & Exper. Neurol. **4**:370, 1945.

Almost half (12 of 25) of these pituitary granulations were in the capsule laterally in the angle between the anterior and posterior lobes. Of the remaining 13, 10 were in the capsule over the convexity of the anterior lobe and 3 over the posterior lobe. Some of these granulations were so large they had compressed the pituitary

TABLE 1.—*Distribution of Sixty Arachnoid Granulations Encountered in Routine Autopsy Material*

Site	Number
Capsule of pituitary gland	25
Infundibulum	2
Choroid plexus	2
Optic nerve	3
Parasagittal region	9
Falx	1
Frontal lobe	7
Parietal lobe	2
Occipital lobe	3
Temporal lobe	5
Spinal cord	1
Total	60

TABLE 2.—*Frequencies of Various Sites of Origin in Two Series of Meningiomas*

Site		Cushing's Series (1938)		Toronto Series (1950)
Parasagittal site		65		46
Dorsolateral convexity				
Anterior	38		37	
Posterior	12	54	26	67
Temporal	4		4	
Sphenoidal ridge and opercular region		55		37
Olfactory groove		29		13
Suprasellar area		28 (including 1 intrasellar)		9 (including 2 intrasellar)
Intraspinal site		18		35
Subtentorial site		16		5
Petrosal site		12		8
Peritorcular site		12		9
Middle fossa		8		5
Falx		7		28
Intraventricular site		4		1
Intraorbital site		1		0
Multiple and combined sites		4		11 (already included above)
Total		313		263

gland, and on this account had been labeled meningiomas. It was only when a whole series was studied that it was realized that they were arachnoid granulations of varying size. All cases were without symptoms referable to lesions in the pituitary gland region and death resulted from the other causes. That these granulations were not meningiomas was also suggested by the patients' average age. It was 57 years, whereas the average age of 25 patients with suprasellar meningioma was 45 years. The average age of 3 patients with intrasellar meningioma was

also 45 years. If tumor properties had been going to develop in these granulations, they would probably have done so earlier in the patients' life. Size and the production of symptoms seem to be the important factors in deciding in favor of calling these granulations meningiomas.

Meningiomas.—There is no close relationship between the histological varieties of meningioma and their sites of origin. The large majority are firmly attached to dura over brain or spinal cord. Very occasionally they may be attached to leptomeninges only, while meningiomas of intraventricular origin are rare. Their most frequent site is in relation to the main dural venous sinuses, especially the anterior half of the superior sagittal sinus. Other common sites are in the Sylvian fissure, the olfactory groove, the tuberculum sellae turcicae, the lesser wing of the sphenoid, the cavernous sinus, and the sheath of the optic nerve. In the posterior part of the cranial cavity they occur near the torcula, near the petrous bone, or near the foramen magnum. With respect to the frequencies of the various sites of origin a series of 263 meningiomas treated on the neurosurgical service of the Toronto General Hospital from 1932 to 1950 is compared with Cushing's series in Table 2. That their distribution is similar to that of the arachnoid villi and cell nests is apparent.

DERIVATION OF COMPONENTS

Endothelial Component.—So many observers have noticed that the cells of this type of meningioma are similar to arachnoid cap cells, there seems little doubt that they are derived from them. In a conclusive case reported by Courville and Abbott [23] the transition between an arachnoid cell cluster and a meningioma was neatly demonstrated.

Fibrous Component.—The origin of this component is more difficult to explain. Either a different tissue participates in the development of the tumor, or the arachnoid cells are or can become fibroblasts. The embryological origin of the arachnoid cell has never been completely settled. Weed [24] thought it was mesodermal, but Harvey and Burr,[25] Harvey, Burr, and van Campenhout,[26] and Raven [27] postulated the theory that it takes origin from the neural crest. The interpretation of the results of the transplantation experiments made in the developing embryo by these workers may not be entirely correct, as it seems more likely that the arachnoid cell is of connective tissue origin. The work of Stone [28] showing that neural crest cells could produce cartilage and connective tissue, and were therefore multipotential, removes this difficulty and makes the controversy pointless, as he regarded the neural crest tissue as "mesectoderm."

Globus [8] believed that the fibrous tissue in meningiomas is of dural origin. Mallory [5] and Penfield [29] regarded the fibroblast as the mature cell being formed

23. Courville, C. B., and Abbott, K. H.: J. Neuropath. & Exper. Neurol. **1**:337, 1942.

24. Weed, L. H.: Contrib. Embryol. **14**:225, 1917.

25. Harvey, S. C., and Burr, H. S.: Development of Meninges, Arch. Neurol & Psychiat. **15**:545 (May) 1926.

26. Harvey, S. C.; Burr, H. S., and van Campenhout, E.: Development of Meninges: Further Developments, Arch. Neurol. & Psychiat. **29**:683 (April) 1933.

27. Raven, C. P.: Arch. Entwcklngsmechn. d. Organ. **134**:122, 1936.

28. Stone, L. S.: Arch. Entwcklngsmechn. d. Organ. **118**:40, 1929.

29. Penfield, W.: Surg. Gynec. & Obst. **45**:178, 1927.

from the cap cell, which can be regarded as an undifferentiated fibroblast. Bailey [30] regarded the surface cell of the arachnoid villus as the mature cell, differentiated as a covering cell, while the fibroblastic tumor had its origin in the fibrous element of the villus. Foot [31] and Kernohan [32] agreed with this concept.

Further insight into the origin of the fibrous tissue and in support of this idea was obtained in a case studied and reported by me.[33] In this case the meningioma consisted of two separate parts closely applied, one endothelial and the other fibrous. The endothelial part formed the outer portion of the tumor and was in contact with dura. It intervened completely between the dura and the inner fibrous portion, so that the fibrous tissue could not have arisen from dura. The arrangement of the tumor, with endothelial cells growing into the dura and covering a fibrous stalk, resembled the normal structure of an arachnoid villus. Tumors appeared to have developed from the two parts at the same time, suggesting that these cells have a common ancestry.

The tissue culture experiments of Bland and Russell [34] showed absence of any cultural distinctions between the different histological types of meningioma and therefore suggested an origin from undifferentiated mesenchymal cells. Such a primitive cell could differentiate into either cap or stalk cell according to conditions. If such a pluripotential cell became neoplastic, both types of tissue might be formed and usually would be intermingled, with one or the other predominating.

Other Types.—That the three main types of meningioma are related to the arachnoid granulation seems well established, and, as these form the majority (almost 90% of the 263 tumors studied), it might be justifiable to restrict the term "meningioma" to them. If the remaining types are also included, the arachnoid granulation may still be of some importance, as both blood vessels and bone can occur spontaneously in the granulation. The ability of the arachnoid cell to ingest particulate matter may be a factor in the development of the so-called melanoblastic and lipomatous growths.

SUMMARY AND CONCLUSIONS

Evidence has been presented that meningiomas take origin in cells of the various types found in the arachnoid villus or in the arachnoid cell clusters in the outer layer of the arachnoid. This evidence is based on the similarity of morphological details and of arrangement bewteen cells of meningiomas and cells of arachnoid villi, and on the correlation between the location of the villi and the favored sites of origin of these tumors.

In every situation where meningiomas have been described, either arachnoid villi or nests of arachnoid cap cells have been demonstrated. This is not surprising when it is realized how ubiquitous these anatomical structures are.

30. Bailey, O. T.: Histologic Sequences in Meningioma, with Consideration of Nature of Hyperostosis Cranii, Arch. Path. **30**:42 (July) 1940.

31. Foot, N. C.: Meningioma, Arch. Path. **30**:198 (July) 1940.

32. Kernohan, J. W.: Tumors of Spinal Cord, Arch. Path. **32**:843 (Nov.) 1941.

33. Wolman, L.: J. Neuropath. & Clin. Neurol., to be published.

34. Bland, J. O. W., and Russell, D. S.: J. Path. & Bact. **47**:291, 1938.

Both meningiomas and arachnoid granulations vary greatly in size. The latter range from microscopic cell nests to hypertrophied granulations as large as the smaller meningiomas. As a result, in some situations it is impossible to be certain whether one is dealing with a true tumor or with a hypertrophied granulation. This is particularly so in the parasagittal region and around the pituitary fossa. In such cases the presence of symptoms is helpful, but if these are absent, size and pressure effects may be the only deciding points in favor of tumor rather than granulation, when a small nodule is found at autopsy.

Dr. E. A. Linell supplied advice, suggestions, and unlimited facilities. Dr. M. I. Tom contributed much useful criticism and help.

Case Reports

MALIGNANT PARAGANGLIOMA ARISING FROM THE ORGAN OF ZUCKERKANDL

Report of a Case with Autopsy Observations

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TUMORS of the chromaffin system have been a subject of great interest both to the morphologist and to the clinician. Excellent résumés are available, and additional cases are being reported at frequent intervals.[1] Hence, it is not our purpose to review the subject of pheochromocytomas in general but rather to focus attention on the group of extra-adrenal chromaffin tumors arising in the organ of Zuckerkandl. Such tumors have been designated by many terms, "paraganglioma" being used only as a convenient device rather than as a term implying a separate histological entity. Our case is a particularly bizarre example, in which distant metastases were demonstrated.

REVIEW OF LITERATURE

The accompanying table lists 14 previously reported tumors of the organ of Zuckerkandl. Nine patients were men and five were women, with an age range of 10 to 71. Six of the tumors gave no clinical evidence of functional activity, while eight caused symptoms of varying significance. Four were found incidentally in patients dying of other diseases; four were clinically diagnosed; the remaining six presented suspicious clinical manifestations but were undiagnosed.

The gross appearance of the tumors was typically that of a spherical, firm mass presenting to one side of the aorta at the level of the inferior mesenteric artery. The tumor was often closely related to the aorta, the inferior vena cava, the ureter, and the sympathetic ganglia. The color was most often brown or grayish-white with varying interstitial bleeding and secondary necrosis. The size varied from 2.5 by 1.5 cm. to 11 by 8 cm. All but one of the tumors failed to produce metastases, although occasional local invasion of the tumor capsule or of the surrounding structures was reported. In one case[2] the tumor was bilateral and

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From the Laboratory of Experimental Oncology, National Cancer Institute, National Institutes of Health, Public Health Service, Federal Security Agency, and the Division of Pathology, University of California School of Medicine.

1. (*a*) Belt, A., and Powell, T.: Chromaffin Cell Tumors of Suprarenal Medulla, Surg., Gynec. & Obst. **59**:9-24, 1934. (*b*) McGavack, T.; Benjamin, J.; Speer, F., and Klotz, S.: Malignant Pheochromocytoma of Adrenal Medulla, J. Clin. Endocrinol. **2**:332-338, 1942. (*c*) Waaler, E.: A Chromaffin Tumour Simulating Graves' Disease, Acta med. scandinav. **123**:1-11, 1945. (*d*) Mackeith, R.: Adrenal-Sympathetic Syndrome, Brit. Heart J. **6**:1-12, 1944. (*e*) Brines, O., and Jennings, E.: Paragangliomas, Am. J. Path. **24**:1167-1188, 1948. (*f*) Jones, C., and McKee, F.: Gastric Paraganglioma with Ulceration, Arch. Path. **48**:570-577 (Dec.) 1949.

2. Cragg, R. W.: Concurrent Tumors of the Left Carotid Body and Both Zuckerkandl Bodies, Arch. Path. **18**:635-645 (Nov.) 1934.

there was accessory adrenal cortical tissue in the retroperitoneal space, as well as a unilateral carotid body tumor. In one case [3] a pheochromocytoma of the right adrenal medulla and a separate adrenal cortical adenoma were found, as well as the paraganglioma. In another case [4] a large pheochromocytoma was primary within the right adrenal medulla and a separate small tumor occurred in Zuckerkandl's organ. Cahill [5] reported an instance in which a large pheochromocytoma arose in the retroperitoneal epigastric region with a separate, smaller tumor at the level of the bifurcation of the aorta.

Fourteen Previously Reported Cases of Tumor of the Organ of Zuckerkandl

	Author	Sex	Age	Evidences of Activity	Clinical Course
1.	Stangl *	M	32	None	Tumor successfully resected
2.	Hausmann and Getzowa †	M	53	Diaphoresis, constipation, cardiomegaly, tachycardia, nervousness, pallor	Tumor unexpected autopsy finding; death from influenzal pneumonia
3.	Handschin ‡	M	45	None	Tumor incidental autopsy finding; death from carcinoma of stomach
4.	Nordmann and Lebkuchner §	M	53	None	Tumor incidental autopsy finding; death from skull fracture
5.	Merkulow ‖	F	26	None	Death followed attempted resection
6.	Reichardt [7a]	F	53	Cephalalgia, diaphoresis, mydriasis, angiospastic retinitis, hypertension of 230/140, cardiomegaly	Sudden death after digitalis and phlebotomy
7.	Cragg [2]	F	39	None	Tumor incidental autopsy finding; death followed surgical treatment for carotid body tumor
8.	Bauer and Leriche ‖	M	40	Paroxysmal pallor, palpitations, nausea, coldness, diaphoresis, cephalalgia, weakness, muscular trembling, paroxysmal hypertension, glycosuria	Tumor successfully resected; paroxysmal attacks relieved but blood pressure constant at 175/115
9.	Gellerstedt [7b]	M	38	Sudden collapse with shock and epileptic attack	At autopsy: cardiomegaly with extensive bleeding into tumor (hyperadrenalism)
10.	Podloucky ¶	M	51	Nausea, emesis, diaphoresis, trembling, exhaustion, cephalalgia, slight hypertension of 160/90	Death by suicide; severe atherosclerosis
11.	Fingerland [3]	M	71	None	Death in diabetic coma; tumor incidental autopsy finding
12.	McCullagh [4]	M	28	Nervousness, tremor, hypertension, glycosuria, tachycardia, elevated basal metabolic rate	Death followed thyroidectomy, due to cellulitis; unexpected autopsy finding
13.	Koster **	F	34	Cephalalgia, nausea, vomiting, diaphoresis, irritability, tachycardia, hypertension, signs of intracranial pressure	Patient entered with a subarachnoid hemorrhage and died soon thereafter
14.	Cahill [5]	F	10	Nervousness, nausea, diaphoresis, hypertension, papilledema, positive benzodioxane test	Death followed attempted resection

* Stangl, E.: Zur Pathologie der Nebenorgane des Sympathicus, Verhandl. deutsch. path. Gesellsch. **5**: 250-255, 1902.

† Hausmann, M., and Getzowa, S.: Ein Paraganglíom des Zuckerkandlschen Organ mit gleichzeitigen Herz- und Nierenhypertrophie, Schweiz. med. Wchnschr. **3**: 889-892, 911-917, 1922.

‡ Handschin, E.: Zur Kenntnis der Zuckerkandl'schen Organe, Beitr. path. Anat. **79**: 728-755, 1928.

§ Nordmann, M., and Lebkuchner, E.: Zur Kenntnis der Paragangliome an der Aortengabel und am Grenzstrang, Arch. path. Anat. **280**: 152-171, 1931.

‖ Merkulow, G.: Ein Fall von einem aus den Nebenorganen von Zuckerkandl entstandenen Paraganglíom, Centralbl. allg. Path. u. path. Anat. **59**: 274-276, 1933.

‖ Bauer, J., and Leriche, R.: Contribution clinique et thérapeutique à l'étude des paragangliomes et des crises d'hypertension adrénalinique, Presse méd. **42**: 1385-1388, 1934.

¶ Podloucky, F.: Ein Phaeochromoblastom des Zuckerkandlschen Organs: Beitrag zur pathologischen Physiologie des chromaffinen Systems, Arch. path. Anat. **306**:372-388, 1940.

** Koster, E.: Adrenal-Sympathetic Syndrome Associated with Paraganglioma of Organ of Zuckerkandl, Ohio M. J. **41**:729-730, 1945.

3. Fingerland, A.: Über zwei Fälle von Phaeochromocytom, Arch. path. Anat. **309**:218-234, 1942.

4. McCullagh, E., and Engel, W.: Pheochromocytoma with Hypermetabolism, Ann. Surg. **116**(1):61-75, 1942.

5. Cahill, G.: Pheochromocytomas, J. A. M. A. **138**:180-186 (Sept. 18) 1948.

Microscopically, the tumors were monotonously uniform. They consisted of large polyhedral cells with oval nuclei and abundant cytoplasm. Giant or syncytial forms were frequent. The nuclei had prominent nucleoli with rare mitotic figures, and fine granulations were noted in the cytoplasm. An abundant capillary blood supply was present, and interstitial hemorrhage was common. Where the epinephrine content was estimated by bioassay, there was lack of correlation between the intensity of the chromaffin reaction and the biological activity of the tumor extract. All of the tumors had either a positive chromaffin reaction, or a positive bioassay for epinephrine, or both.

Only one paraganglioma has been reported to have spread by metastasis. It was observed by Halscheidt.[6] The tumor arose from the hilum of the left kidney. The patient was a 24-year-old white man who died after extirpation of the primary lesion. Metastases, of a slightly less differentiated pattern, were found in the skull and in regional lymph nodes. The tumor gave evidence of function, manifested by typical paroxysmal attacks of hyperadrenalism with sustained arterial hypertension. All other reported malignant tumors of this type have originated from the adrenal medulla. McGavack,[1b] in reviewing this group, accepted eight as being well substantiated. None of the eight tumors produced symptoms indicating a physiological function and five were bilateral.

REPORT OF CASE

E. H., a 76-year-old white woman, a widow, entered Laguna Honda Home on Dec. 14, 1945. She was admitted because of inability to care for herself and had no specific symptoms. Her past history and a complete system review were noncontributory. One sister had died of gastric carcinoma.

Physical Examination.—The blood pressure was 210/105; the respiration rate, 20, and the pulse rate, 82. The patient was obese, with a florid face and no apparent distress. Head, eyes, neck, lymph nodes, and lungs were normal. The heart was enlarged 2 cm. lateral to the midclavicular line; the cardiac rhythm was regular, and the sounds were clear. Abdominal palpation disclosed nothing remarkable, and neurological examination indicated no abnormality.

Laboratory Studies.—The urine showed only a faint trace of albumin. There were occasional white blood cells in the centrifuged sediment. Mazzini and Kolmer serologic tests were negative for syphilis.

Clinical Course.—During the first two years the patient had occasional periods of nausea and retching. A single blood pressure determination was reported as 180/88.

In 1948 intermittent episodes of dizziness, occasionally associated with nausea, vomiting, and syncope, became more prominent. In February the blood pressure was 210/105; in April it was 270/80, and repeated syncope was noted. Examination showed an enlarged heart and multiple extrasystoles. By June low back pain appeared, together with urinary frequency and urgency.

In January, 1949, a globular mass was palpated within the abdomen just to the left of the umbilicus. By October the patient was falling more frequently and had severe dizziness when standing. The blood pressure was then 195/90. A urinalysis gave negative results. The blood hemoglobin was 15.5 gm., and the red blood cell count was 4,600,000. The white cell count was 14,150, with 76% mature neutrophils. The blood nonprotein nitrogen was 44 mg. per 100 cc. Roentgenographic studies of the abdomen, stomach, small intestine, large intestine, and kidneys failed to demonstrate abnormalities.

On March 21, 1950, the patient suddenly became unconscious for a brief period. The skin was cold and clammy, the pulse rate was 88, and the lungs were clear. In November the patient began a steady downhill course. She would respond only to painful stimuli. The right pupil

6. Halscheidt, W.: Klinische und pathologisch-physiologische Betrachtungen an Hand eines extrasuprarenalen malignen Paraganglioms, Ztschr. ges. inn. Med. **4**:116-123, 1949.

reacted sluggishly, the left briskly. Dependent lung areas showed decreased breath sounds and scattered *rales*. Slight left-sided heart enlargement was noted, and a distant regular rhythm. The blood pressure was, right arm, patient reclining, 170/86, left arm, patient reclining, 164/88. A large mass filled the lower left side of the abdomen; it was fixed and firm, with irregular contours. Neurological examination showed hyperactive deep tendon reflexes. The nonprotein nitrogen was 185 mg. per 100 cc. The patient died on Nov. 27, 1950, at the age of 81.

Autopsy.—A retroperitoneal tumor was found in the abdomen, anterior to and slightly to the left of the bifurcation of the aorta. It had a firm, lobulated surface and measured 12 by 5 by 5 cm. The mass was adherent to the aorta and the inferior vena cava and crossed the midline at its greatest width. It encircled the inferior mesenteric artery, without obstructing the vessel, with its greatest mass

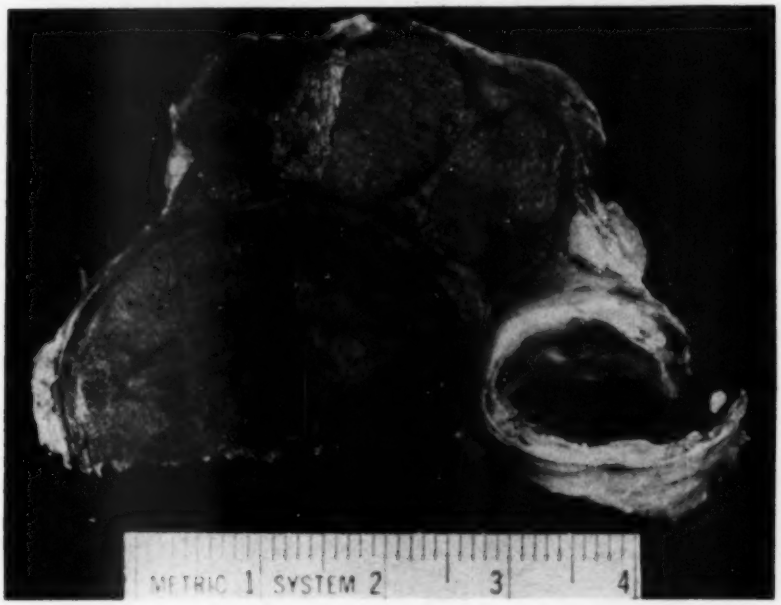

Fig. 1.—Representative cross section of the tumor. ¡Note the lobulated pattern of dark tumor tissue with an apparently intact heavy capsule. The inferior mesenteric artery is partially surrounded, and the aorta shows severe sclerosis and adheres to the tumor tissue; × 2.5.

at the level of this artery. The left ureter was laterally deviated and adherent to the tumor capsule but was not obstructed. The tumor extended inferiorly over the bifurcation of the aorta and along the left iliac artery. The major branches of the sympathetic ganglia on the left were also closely related to capsular tissue. The regional lymph nodes were slightly enlarged but showed no gross abnormality. On cut surface the mass consisted of deep brown lobulated tissue within a circumscribed fibrous capsule (Fig. 1).

Two separate masses of similar tissue were found in the retropleural space at the insertions of the left fourth and eighth ribs. Each mass measured 2 cm. in diameter and was adherent to vertebral column, rib, and intercostal muscle.

The heart weighed 300 gm. and had a globular, hypertrophied appearance. The wall of the left ventricle was 2.0 cm. in thickness. The smaller branches of the coronary arteries showed severe sclerosis without complete occlusions. The aortic valve also had a severe arteriosclerotic thickening and opacity. The adrenals were normal

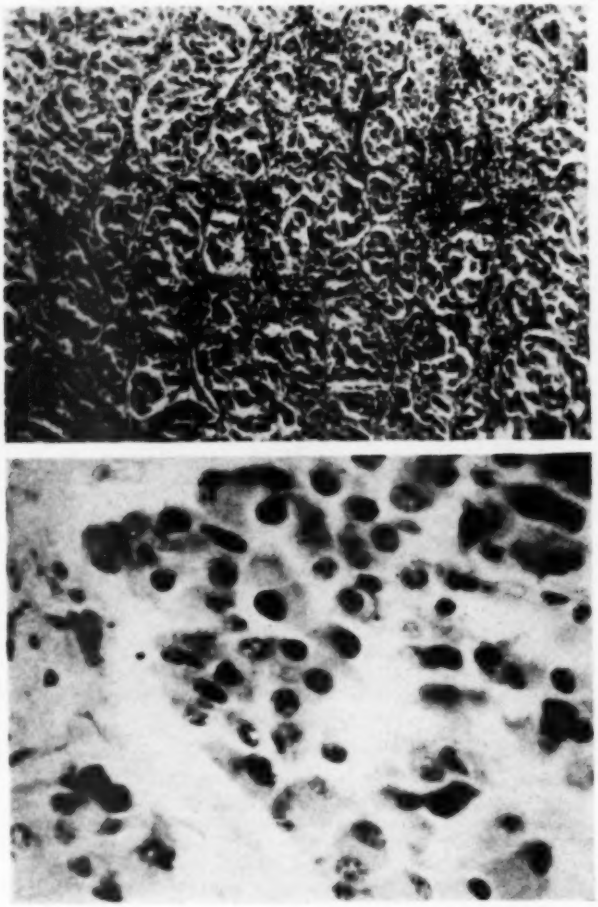

Fig. 2.—Upper part: Histological pattern of the primary tumor. The cells, large, regular, show an alveolar-like pattern. There is recent hemorrhage involving the stroma. Hematoxylin and eosin; × 100.

Lower part: High-power magnification of the primary tumor. The cells occasionally show irregular processes. The cytoplasm is abundant, with syncytial forms and oval nuclei. Mitoses are absent. Hematoxylin and eosin; × 460.

in weight but showed a thick, nodular cortex. The kidneys together weighed 200 gm. and showed severe sclerosis of the major arteries with numerous small cortical scars. Aortic atherosclerosis and calcification were far advanced in the

abdomen but relatively slight in the thorax. The brain weighed 1,200 gm. and showed severe sclerosis of the basal vessels. The cortex presented moderate atrophy with symmetrical dilatation of all ventricles. Small cystic lesions were noted in the caudate nuclei. No other positive changes were noted except for pulmonary congestion and involutionary changes of the internal genitalia.

Microscopic Observations.—The tumor was composed of masses of large polyhedral cells containing abundant, slightly basophilic, finely granular cytoplasm. The nuclei were oval, with fine chromatin and numerous small nucleoli. The cells had irregular cytoplasmic processes with indefinite cell margins, and frequently

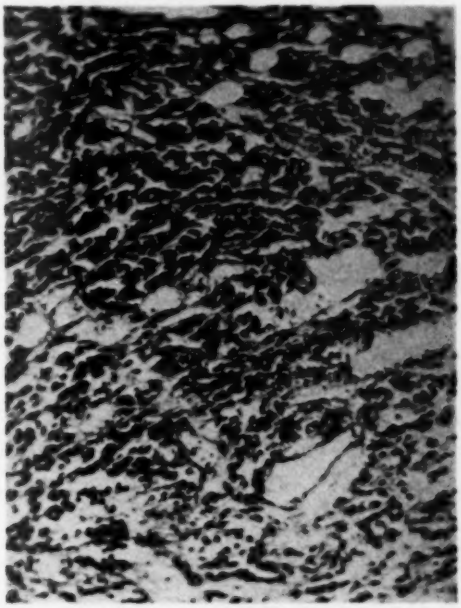

Fig. 3.—Section taken from retroperitoneal fat tissue. The tumor has penetrated its capsule and is diffusely replacing the nearby fat. The tumor pattern remains essentially unchanged. Hematoxylin and eosin; × 100.

there were syncytial forms. Occasionally, the nuclei had larger, pleomorphic shapes. Mitotic figures could not be found, and the cells in general were quite regular. The cells were arranged in small clumps or masses with a pseudoalveolar pattern and separated by delicate capillaries and abundant collagen (Fig. 2, lower part). There were many areas of hemorrhage and necrosis, while other areas showed dense masses of hyaline collagen and secondary calcification (Fig. 2, upper part). Staining with azure-eosin showed diffuse brown pigmentation of the cytoplasm. Mallory-Heidenhain azocarmine stains demonstrated an abundance of blue cytoplasmic granules; only the intercellular fibrous tissue gave a positive collagen or reticulum reaction. Iron stains revealed sparse granules within stromal macrophages.

Although a dense capsule was present, at numerous points the tumor had completely penetrated it and was extending within the retroperitoneal fat (Fig. 3). Well-differentiated cells frequently invaded the perivascular and perineural lymphatics (Fig. 4, upper part). The regional retroperitoneal lymph nodes contained

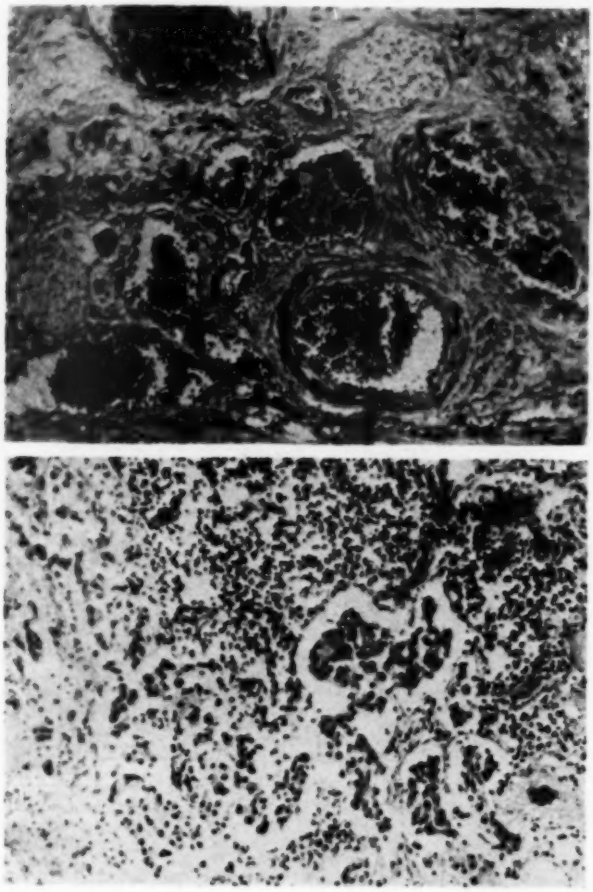

Fig. 4.—Upper part: An area close to the capsule. Small masses of tumor are invading the abundant perivascular and perineural lymphatics. Hematoxylin and eosin; × 100.

Lower part: Metastatic deposit in a regional lymph node. The tumor has penetrated both lymphatics and parenchyma of the lymph node. Occasional large syncytial forms are present. Hematoxylin and eosin; × 100.

small metastatic deposits of identical tumor (Fig. 4, lower part). The retropleural tumors showed an identical pattern, with similar invasion of local structures, including skeletal muscle.

The arterioles revealed a diffuse, severe hyaline sclerosis and a "fibrinoid" type of smudging, especially notable in the heart, pancreas, spleen, brain, and kidneys (Fig. 5). The larger elastic arteries had advanced calcific arteriosclerosis, while the intermediate vessels showed muscular and elastic tissue hypertrophy. No acute arteritis was found, and the renal cortex showed only occasional glomerular fibrosis.

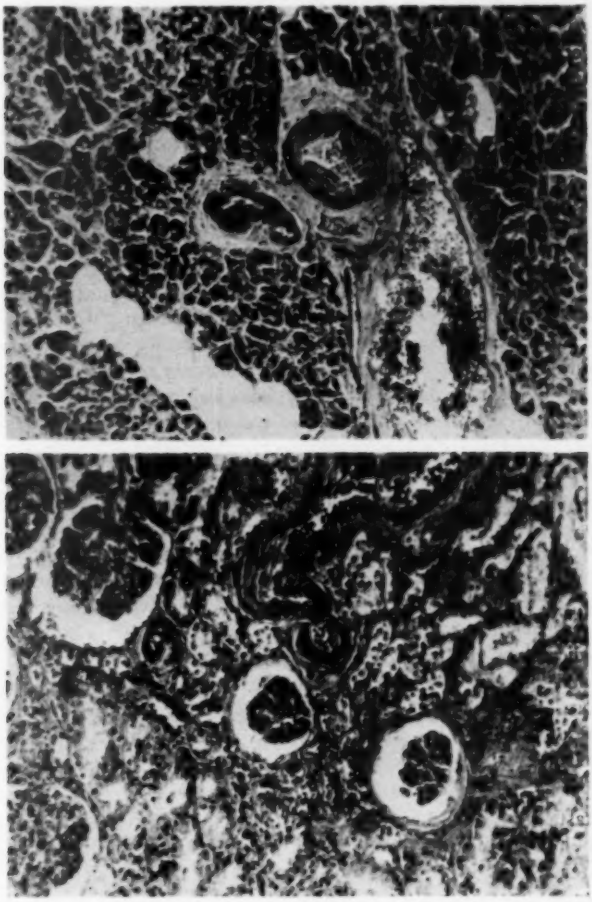

Fig. 5.—Upper part: Vascular lesions within the pancreas. An arteriole shows severe hyalinization, with eosinophilic smudging of the vessel wall. A slightly larger muscular artery shows thickening and hypertrophy. Hematoxylin and eosin; × 100.

Lower part: Vascular lesions within the renal cortex. Severe arteriolosclerosis and eosinophilic necrosis of the vessel are present. The glomeruli show moderately advanced ischemic changes. Hematoxylin and eosin; × 100.

The remaining positive findings were: myocardial hypertrophy, nodular adrenal cortices, hyalinized uterine leiomyomata, and mixed senile-arteriosclerotic brain changes.

The final diagnosis was: malignant chromaffin tumor of the organ of Zuckerkandl with abdominal and pleural metastases, myocardial hypertrophy, severe generalized arteriosclerosis and arteriolosclerosis, nodular hyperplasia of the adrenal cortices, and uterine leiomyomata.

COMMENT

The gross and microscopic structure of the tumor clearly identifies it with those previously observed arising from the organ of Zuckerkandl. The striking deviation is its cancerous character without associated loss of differentiation. Although the clinical studies are not entirely convincing, the assumption appears warranted that a vasopressor substance was produced by the tumor. McGavack and associates [1b] could find no acceptable instances of functioning ability in malignant pheochromocytomas while the only instance of a functioning malignant paraganglioma was reported by Halscheidt.[6] In this regard it may be significant that the present tumor is the largest paraganglioma yet observed and that even the metastases were remarkably differentiated. These features, and the advanced age of the patient, suggest a relatively slow-growing neoplasm.

This case suggests several other conjectures. The mechanism of death remains unexplained. Even in the absence of surgical intervention pheochromocytoma has been associated with sudden demise.[7] Terminal azotemia and acidosis were prominent in the present case. To what degree vasospasm or dehydration contributed to this picture is difficult to say, as the morphological findings were quite inadequate.

In regard to the pattern of metastatic tumor observed in the present case, it may be suggested that the retropleural tumor masses represent multicentric growth. In view of the extensive permeation of lymphatics and lymph nodes and the identical histological aspect, however, it would appear more likely that they are true metastases.

The vascular changes in reported cases have always excited interest. In this particular case the significance of the vascular degenerative disease is obscured by the age of the patient. The vascular lesions were characteristic of a severe, sustained vascular hypertension. The example reported by Cahill [5] of a 10-year-old girl indicated an accelerated atheromatosis in the tumor's presence.

As a final comment, the term "paraganglioma" was used by Jones and McKee [1f] in the sense of a benign, nonfunctioning tumor. Since the present case does not satisfy these qualifications, the question of terminology is left to those more experienced in semantics.

SUMMARY

A metastasizing pheochromocytoma is reported as arising from the organ of Zuckerkandl in an 81-year-old woman. The clinical and morphological findings are consistent with a functioning tumor.

7. (*a*) Reichardt, R.: Chromaffiner Tumor des Zuckerkandlischen Organes und innere Sekretion, Endokrinologie **14**:180-186, 1934. (*b*) Gellerstedt, N., and Thyresson, N.: Zwei seltene Tumoren des Sympathicus, Upsala läkaref. Förh. **44**:303-316, 1938. (*c*) Dolgin, W.: Pheochromocytoma and Sudden Death Following Injury of the Head, Arch. Path. **40**:135-140, (Aug.) 1945. (*d*) Fingerland.[8]

Laboratory Methods and Technical Notes

STUDIES ON THE METHODS OF STAINING THE ISLET CELLS OF THE PANCREAS

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Cytological studies of the islets of Langerhans of the pancreas have required the use of many and varied histological techniques.[1] Each method has had its advantages and disadvantages, but the most serious deficiencies have been the inexact chromatic specificity and the lack of correlation between the various methods. The relatively small number of cellular structures differentially

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1. (*a*) Bensley, R. R.: Studies of the Pancreas of the Guinea Pigs, Am. J. Anat. **12**:297-388, 1911-1912. (*b*) Cajal, S. R.: Algunas variaciones fisiológicas y patológicas del aparato reticular de Golgi, Trab. lab. inv. biol. **12**:127-227, 1914. (*c*) Bowie, D. J.: Cytological Studies of the Islets of Langerhans in a Teleost, Neomaenis Griseus, Anat. Rec. **29**:57-73, 1925-1926. (*d*) Bayley, J. H.: Staining Methods for the Islets of Langerhans, J. Path. & Bact. **44**:272-276, 1937. (*e*) Saguchi, S.: Cytological Studies of Langerhans' Islets, with Special Reference to the Problem of Their Relation to the Pancreatic Acinus Tissue, Am. J. Anat. **28**:1-58, 1920-1921. (*f*) Van Campenhout, E.: Étude sur le développement et la signification morphologique des ilots endocrines du pancréas chez l'embryon du mouton, Arch. biol. Liége **35**:45, 1925; (*g*) Contribution à l'étude de l'histogenèse du pancréas chez quelques mammifères: Les complexes sympathico-insulaires, ibid. **37**:121-171, 1927; (*h*) Argentaffin Cells of the Pancreas, Proc. Soc. Exper. Biol. & Med. **30**:617-618, 1933; (*i*) Les relations nerveuses de la glande interstitielle des glandes génitales chez les mammifères, Rev. Canad. Biol. **8**:374-429, 1949. (*j*) Neubert, K.: Bau und Entwicklung des menschlichen Pankreas, Arch. Entwcklngsmech. Organ **111**:29-118, 1927. (*k*) Ludford, R. J., and Cramer, W.: Secretion and the Golgi Apparatus in the Cells of the Islets of Langerhans, Proc. Roy. Soc. London, s.B **101**:16-24, 1927. (*l*) Beams, H. W.: Golgi Apparatus, Canalicular Apparatus, Vacuome, and Mitochondria in the Islets of Langerhans of the Albino Rat, Anat. Rec. **46**:305-327, 1930. (*m*) Bloom, W.: A New Type of Granular Cell in the Islets of Langerhans of Man, ibid. **49**:363-371, 1931. (*n*) Simard, L. C.: Les complexes neuro-insulaires du pancréas humain, Arch. anat. micr. **33**:49-64, 1937. (*o*) Ferner, H.: Über die Entwicklung der Langerhansschen Inseln nach der Geburt und die Bedeutung der versilberbaren Zellen im Pankreas des Menschen, Ztschr. mikr.-anat. Forsch. **44**:451-488, 1938. (*p*) Gomori, G.: Studies on the Cells of the Pancreatic Islets, Anat. Rec. **74**:439-459, 1939; (*q*) A Differential Stain for Cell Types in the Pancreatic Islets, Am. J. Path. **15**:497-499, 1939; (*r*) Observation with Differential Stains on Human Islets of Langerhans, ibid. **17**:395-406, 1941; (*s*) Pathology of the Pancreatic Islets, Arch. Path. **36**:217-232 (Aug.) 1943. (*t*) Richardson, K. C.: The Influence of Diabetogenic Anterior Pituitary Extract on the Islets of Langerhans in Dogs, Proc. Roy Soc., London, s.B **128**:153-168, 1948. (*u*) Groberty, J.: Contribution histologique a l'étude du diabète expérimental: Le diabète par l'alloxanne, Bull. histol. appliq. à la physiol. **25**:8-13, 1948. (*v*) Cutting, W. C., and Laqueur, G. D.: Alloxan Diabetes: Cellular Changes and Inhibitory Actions of Colchicine, Stanford M. Bull. **4**:48-51, 1946.

stained by the more generally applicable of the techniques used to distinguish the different types of islet cells has also been a great disadvantage.[2]

The present study was undertaken with a view to reassessing the various technical methods employed in the past, with the hope of being able to select a small group of techniques the application of which would reveal the minute details of the cytological structure of the islets of Langerhans with uniformity and precision. In the course of this work, which extended over a period of four years, most of the techniques of fixation and staining that have been described and recommended in the literature of the last 60 years were carefully carried out on the pancreatic tissues of man and a variety of animal species. The various species to the tissues of which the histological techniques were applied included large numbers of rabbits of various ages, and considerable numbers of the common laboratory and domestic mammalian species. Smaller numbers of birds, reptiles, and fishes were also studied.

As a result of this exhaustive survey, several methods described in the literature have been modified and combined to provide a relatively small group of simple techniques which, if carefully applied, yield the desired results with consistency. These methods are suitable for the detailed cytological study of the embryonic or the adult pancreas and the normal or the pathological pancreas. It is to be emphasized, however, that the techniques which it is the purpose of this paper to describe will yield completely satisfactory results only if care is exercised at every stage of the method, since the best of staining procedures cannot retrieve the damage of poor fixation or incomplete dehydration. For this reason, the technical methods suitable for the study of adult or embryonic pancreatic tissues are presented in detail.

METHODS

Embryos are killed by immersing the open uterus in the fixative. A few minutes after immersion a small perforation should be made in the amniotic sac with scissors. This allows the embryo to come into contact with the fixative while retaining its placental attachment. The attached placenta is most useful for manipulating the embryo. Newborn animals are killed by decapitation. Adult animals may be killed by air embolism, a blow on the nape of the neck, or by intravenously injected pentobarbital sodium. Each one of these methods has its obvious advantages and disadvantages. Air embolism should be avoided when killing pregnant rabbits because the air bubbles pass into the embryonic sac and render the subsequent treatment of the embryos difficult.

Samples of pancreas should be removed immediately after death. Embryos younger than 15 days since mating of their progenitors should be fixed and processed *in toto*. The pancreas of embryos between 15 and 20 days old are dissected as soon as the surfaces of the embryos are hard enough to permit complete horizontal section of the abdomen, or in about one-half to one hour after fixation begins. As the age of the embryo increases, the abdomen is opened as soon as possible, in order to expose the pancreas to the fixative. The pancreas is removed together with the neighboring organs and placed in fresh fixative. Pancreases of newborn and adult animals must be dissected and removed with extreme gentleness. Specimens from animals such as the rabbit with a diffuse pancreas are fixed in large pieces, while those from animals with a compact pancreas (the dog) should be trimmed in the fresh state into blocks about 3 mm. thick.

Routine Procedures for the Study of the General Cytological Aspect of the Pancreatic Islets

1. Fix tissue for 8 to 16 hours in Zenker-formol. Zenker's solution is made up as follows:

Mercury bichloride	5.0 gm.
Potassium bichromate	2.5 gm.
Sodium sulfate	1.0 gm.
Distilled water	100 cc.

To 80 cc. of the above solution add 20 cc. of 40% formaldehyde (formalin) neutralized with sodium carbonate or marble chips. The formalin must be neutral when tested with litmus paper, and it should be added just before use. It is better that the tissues be placed in the dark while they are fixed in this mixture.

2. Wash fixed tissues in running water for 24 hours or more.

2. Bloom,[1m] Gomori.[1p]

3. (*a*) Dehydrate the tissues from animals 1 week of age or older as follows:

95% alcohol containing 0.5% iodine	24 hours
Dehydrated alcohol used twice	8 hours
Dehydrated alcohol used once	16 hours
Dehydrated alcohol	8 hours
Dehydrated alcohol	16 hours

Shake bottles at least four times a day.

(*b*) Dehydrate tissues from embryos and animals younger than 1 week of age according to the following schedule:

Alcohol 40%	2 hours
Alcohol 50%	2 hours
Alcohol 60%	2 hours
Alcohol 70%	2 hours
Alcohol 80%	3 hours
Alcohol 90%	3 hours (with 0.25% iodine)
Alcohol 95%	10 hours (with 0.25% iodine)
Dehydrated alcohol	3 hours
Dehydrated alcohol	5 hours

4. (*a*) Clear tissues from animals 1 week of age or older with toluol in the following manner:

Toluol used twice	8 hours
Toluol used once	16 hours
Toluol, pure	8 hours
Toluol, pure	16 hours

(*b*) Clear tissues from embryos and tissues from animals younger than 1 week of age according to the following scheme:

Toluol—dehydrated alcohol 1:1	2 hours
2:1	2 hours
Toluol used twice	3 hours
Toluol used once	10 hours
Toluol, pure	2 hours
Toluol, pure	4 hours

5. (*a*) Embed tissues from animals older than 1 week in a mixture of paraffin (melting point, 50-52 C.) and raw, thoroughly dry beeswax (yellow wax, U. S. P.) (9:1) in paraffin ovens at 54-56 C. as follows:

Paraffin oven No. 1	8 hours
Paraffin oven No. 2	16 hours
Paraffin oven No. 3	1-5 days

It has been found that the adequate elimination of toluol from the tissue requires the use of three ovens.

(*b*) Place embryos and tissues from animals younger than 1 week in a mixture of toluol and paraffin (1:1) for two hours and then treat as adult tissues.

6. Block in ice water, using metal boats.

7. Cut at 2.5-5 or 7.5 microns (μ) as desired.

8. Mount sections by the gelatin method and dry at 45 C. in an oven containing some formol vapor.

Masson Trichrome Stain Applied as a General Cytological and Differential Stain for the Pancreas.[3a]

1. Hydrate.
2. Treat with iodine-alcohol 1% for 10 to 30 minutes.
3. Rinse with distilled water.
4. Treat with sodium hyposulfite 5% solution for 1 minute.
5. Wash in tap water for 5 to 15 minutes.

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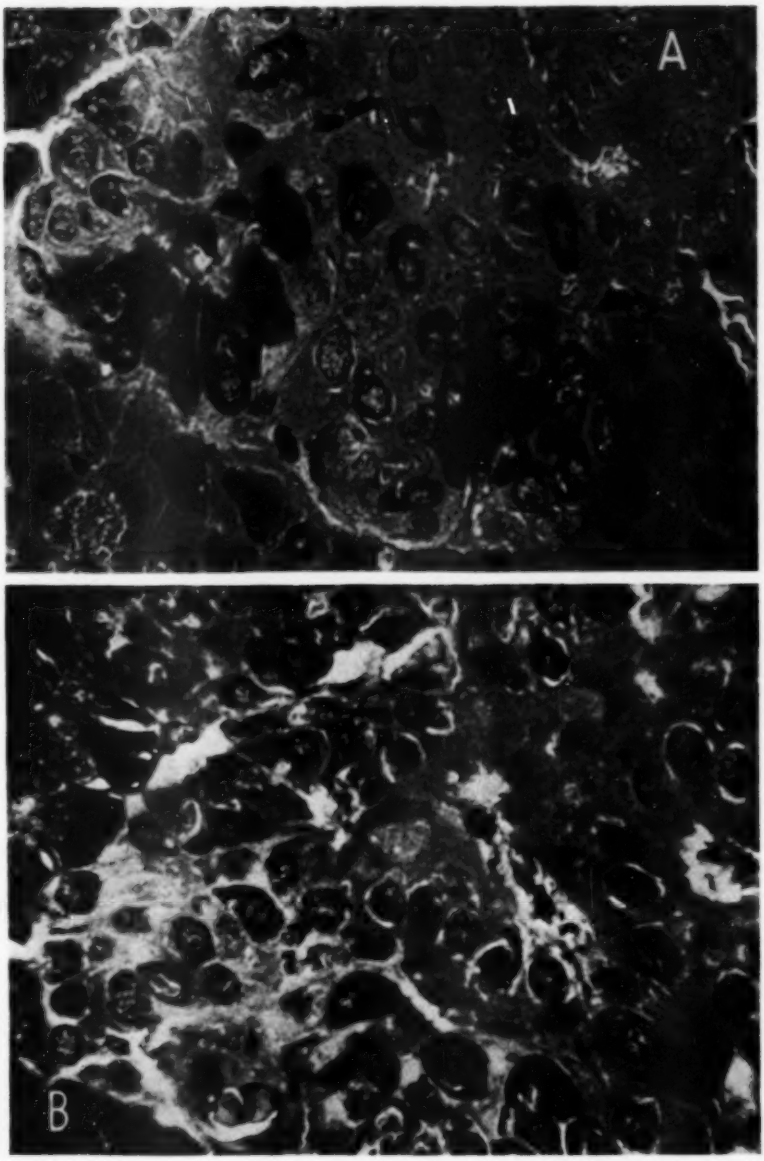
3. Masson, P.: (*a*) Diagnostics de laboratoire, Paris, Norbert Maloine & Fils, 1923; (*b*) Histogénèse des neurofibromes cutanés diffus, Bull. soc. franç. dermat. et syph. **42**:1278-1293 (July) 1935.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6. Rinse well with distilled water.

7. Treat with iron-alum (iron and potassium aluminum sulfate) 5% for 5 minutes to 10 hours at 52-56 C.

8. Rinse cautiously in distilled water.

9. Stain in Regaud's hematoxylin for 5 minutes to 10 hours at 52-56 C.

10. Rinse with 95% alcohol until no more color comes out.

11. Differentiate at 52-56 C. in picric-alcohol solution (2 parts of a saturated solution of picric acid [trinitrophenol] in 95% ethyl alcohol diluted with 1 part of 95% alcohol) until chromatin is sharply differentiated. Control this step under the microscope by rinsing with 95% alcohol. There is some advantage in slightly overdifferentiating the nuclei.

12. Wash in running water until no more picric acid is present, 20 to 40 minutes or more according to the thickness of the section.

13. Stain at least one hour with a ponceau-fuchsin mixture, comprising 4 parts of 1% ponceau,[4] 1 part of 1% acid fuchsin,[4] and 1 part of 1% fuchsin S,[5] all diluted 1:10 in 1% acetic acid water. Twelve hours of staining produces excellent results.

14. Rinse briefly in 1% aqueous acetic acid.

15. Differentiate in a 5% phosphomolybdic acid solution until the delta cells are unstained or pale gray. The time varies, depending on the brand of ponceau, but is approximately 2 to 10 hours at 52-56 C. Check with the microscope, using distilled water.

16. When delta cells and other cyanophilic structures are thoroughly differentiated, rinse well with distilled water.

17. Apply aniline blue made according to Masson's original method, for 10 to 20 minutes. A subdilution of this stain 5:100 in 1% acetic acid is more convenient in controlling the staining process. It is applied for 2 to 6 hours while controlling the process under the microscope until the desired effect is obtained.

18. Rinse briefly with distilled water.

19. Differentiate the aniline blue with 1% aqueous phosphomolybdic acid for 10 to 45 seconds.

20. Rinse in 1% acetic acid and place in acetic acid 1% for 5 to 30 minutes until all colors and structures are clearly defined.

21. Dehydrate with dehydrated alcohol (absolute alcohol) (avoid dilute alcohol) by the dropper method or by using three changes of dehydrated alcohol for 2 to 5 seconds, and 1 minute, respectively.

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4. The brand used was R. A. L., Kuhlmann, Paris; sold by Pierre Mercier, 312 Sherbrooke St., East Montreal, Que., Canada.

5. If fuchsin S is not available, the ponceau-fuchsin mixture may be made as follows: ponceau 1%, 4 parts, and acid fuchsin 1%, 2 parts. Use as described above.

EXPLANATION OF FIGURE 1

A, photomicrograph of part of a pancreatic islet from a rabbit 1 year old, stained by the trichrome technique. Alpha cells (red-brown) appear granular and almost black. Their Golgi apparatus can be seen as a negative image. Beta cells (pink) appear gray and are faintly granular. They constitute the greater part of this islet. Their Golgi net also appears as a negative image. Delta cells (sky-blue) appear a faint or pale gray color. They are agranular and may be seen clustered around the group of alpha cells located at the lower left part of the islet.

The acinar tissue shows a minimal amount of retraction, and cell borders are indistinct. Zymogen granules are coarse and dark.

This section is cut at 2.5 μ. × 1,200.

B, photomicrograph of an islet from the same block of tissue shown in *A*, stained by the Gomori technique. Alpha cells (red) and beta cells (steel blue) are almost indistinguishable in the photograph, although some of the alpha cells are darker gray. A cluster of delta cells (pale gray-pink) may be seen clearly at the lower left corner of the islet. Pale areas in these cells that photograph as vacuoles represent the macular zone. The section is 2.5 μ in thickness. × 1,200.

22. Clear with three changes of toluol for 5 minutes each. Rinse with fresh toluol before mounting in Canada balsam or permount,* dry on a hot plate at 52-58 C. for 24 hours. Use No. 0 or 1 cover slips and cover slip weights.

Results.—When the sections are thin (2.5 μ) the granules of the alpha cell are seen individually. They are stained in deep fuchsin, fuchsin-brown or brown-lilac. These three varieties of granular staining of the alpha cell may coexist in the same islet. Mitochondria cannot be differentiated from alpha cell granules. The Golgi apparatus appears as a juxtanuclear vacuole. A cyanophilic macular structure of irregular shape and position is clearly demonstrated.

Beta cell granules stain salmon-pink. Mitochondria stain red-brown or fuchsin-red but are not always easy to distinguish from the granules. However, when these structures are segregated, the color difference is striking. When the cytoplasm can be seen between granules, it has a pale grayish tint. The Golgi net of the beta cells appears as a series of branched clear channels. Beta cells also show a cyanophilic structure similar to that of the alpha cell. Occasionally beta cells show, on one edge a cuticula that is brilliantly stained red-orange.

The delta cells do not possess granules but have a glassy sky-blue cytoplasm with small rod-shaped red mitochondria. The Golgi net of the delta cell appears as a clear canal that is moderately branched. A cyanophilic macular structure is also present, and it can be distinguished from the rest of the blue cytoplasm by its higher degree of refractility.

Ductular epithelium, centroacinar cells, goblet cells, and the cells of the serous glands all have their cytoplasm colored an indifferent pale blue-gray, and show red mitochondria. The material accumulated in the small ducts stains red, but in the larger ones it appears dirty blue.

In addition to typical alpha, beta, and delta cells, the walls of the secretory ducts contain certain cells that stain in different ways, varying from completely unstained "clear cells" to those that are very dark brown.

Acinar cells are lilac at their basal region and possess red mitochondria and red zymogen granules. Many shades of lilac can be seen in the acinar cells. Collagen, mucin, hyalin, and colloid are all stained various shades of blue. Ganglion cells stain brown-gray and show various types of green and brown pigment. Schwann cells are stained red.

Nuclear chromatin stains deep black and is shown with great delicacy. Nucleoli are red.

In embryos, the results are similar. It is impossible to detail in this paper all of the results obtained with embryonic material, but it may be noted that the differentiation of the various islet cells and the peripheral nervous system is so clear that early immature types of islet cells can be studied in more detail than has ever been possible before.

Gomori's[19] Chromic Hematoxylin-Ponceau Stain

1. Carry out steps 1 to 5, inclusive, of the trichrome method.
2. Treat with potassium permanganate (Gomori's original formula) for 1 to 2 minutes.
3. Wash in running tap water for 1 minute.
4. Bleach in potassium meta-bisulfite 5% for ½ to 1 minute.
5. Wash in running tap water for 5 minutes.
6. Stain in chromic hematoxylin (Gomori's original formula) for 15 to 45 minutes at room temperature, or for 5 to 15 minutes at 52-56 C.
7. Rinse with 95% alcohol until no more color washes out.
8. Differentiate in 1% solution of reagent hydrochloric acid in 95% alcohol for 1 minute.
9. Wash in tap water for 5 to 10 minutes.
10. Stain in ponceau-fuchsin (the same staining mixture that is used in the trichrome method) for 15 to 45 minutes.
11. Rinse in 1% acetic acid.
12. Differentiate with phosphomolybdic acid 1% until alpha and beta cells are clear (approximately 5 to 30 minutes depending on the ponceau mixture).
13. Rinse with 1% acetic acid and place in 1% acetic acid for 1 minute.
14. Dehydrate, clear, and mount as before.

Results.—Alpha granules are deep red. Beta cell granules are steel blue to black; the mitochondria of beta cells are pale red; the cytoplasm of beta cells is pale gray. The cytoplasm of the delta cells is amphophilic, appearing pale gray to gray-orange. Mitochondria of beta and delta cells are pale orange-red.

Ductular epithelium, centroacinar cells, goblet cells, and cells of the serous glands all stain an indifferent pale gray to gray-orange. The mucin of goblet cells stain deep steel blue.

Basophilic substance in the acinar tissue appears bluish purple, and zymogen granules are red-orange.

In embryonic tissue the method permits a moderately good degree of differentiation of immature islet cells, particularly of the alpha cells, but it is not as useful as the trichrome method.

Procedure for the Demonstration of the Mitochondria of the Islet Cells

Schridde's Method.[6]—The procedure is carried out as follows:

1. Fix as follows:

Müller-formol	2 days
Müller solution	2 days
Osmic acid 2%	2 days

The formol must be 40% formaldehyde and neutralized as was that used in the preparation of Zenker-formol. Prepare the Müller-formol when it is to be used. Tissues from embryos and adult animals are fixed similarly.

2. Wash in running water for 24 hours.

3. Employ the same dehydrating and embedding techniques used previously. Do not add any iodine to any of the alcohols.

Staining with Iron Hematoxylin.—

1. Hydrate.

2. Treat with iron-alum 5% for 6 to 10 hours at 52-56 C., or for 1 to 2 days at room temperature.

3. Treat with Regaud hematoxylin for 6 to 10 hours at 52-56 C., or for 1 to 2 days at room temperature.

4. Rinse briefly with distilled water.

5. Differentiate with iron-alum 1% until mitochondria are distinct (approximately 5 to 60 minutes).

6. Rinse well with distilled water.

7. Wash in three changes of distilled water for 60 minutes each.

8. Dehydrate, clear, and mount as before.

Results.—Mitochondria and zymogen granules are black. The granules of beta cells are gray. The results are excellent in older embryos and in younger animals.

Procedure for the Demonstration of the Golgi Apparatus of the Islet Cells

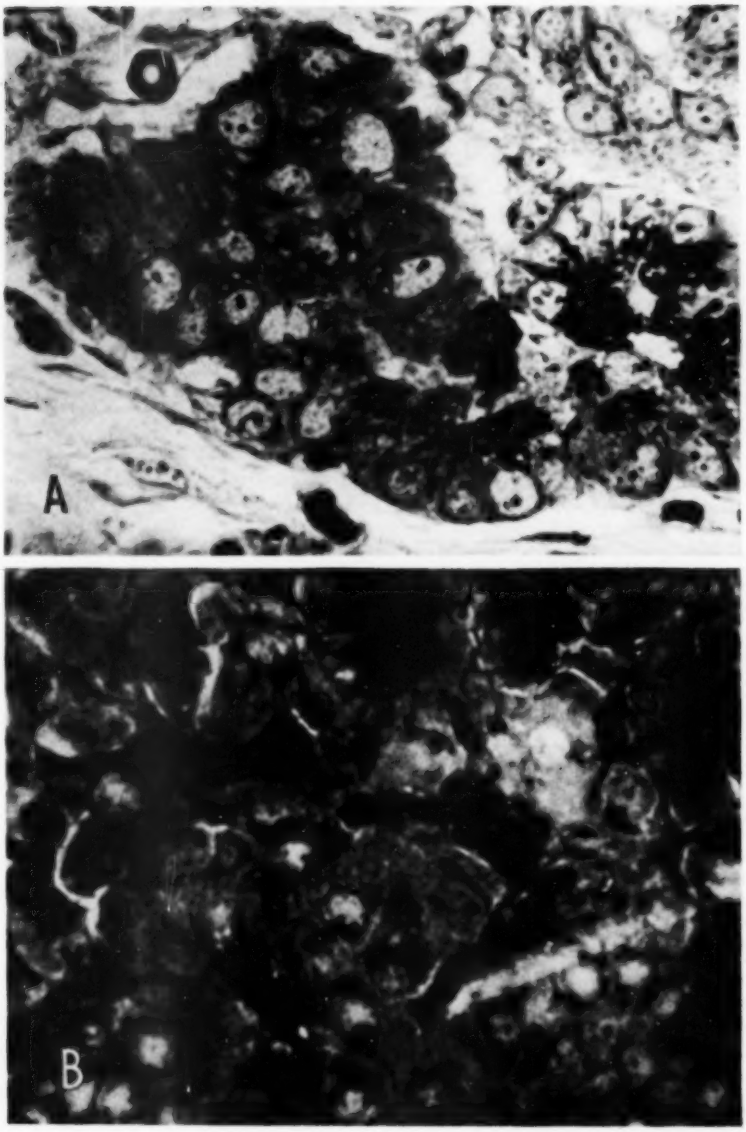
Silver Method of Aoyama.[6]—The procedure follows:

1. Fix for 5 to 7 hours in a solution of

Cadmium chloride	1 gm.
Distilled water	85 cc.
Neutral formol (40% formaldehyde)	15 cc. (add the formol before using)

2. Rinse three times in distilled water quickly and transfer to a solution of $AgNO_3$ 2%, in the dark for 9 to 12 hours. It is advisable to shake the specimen a few times during this procedure.

6. Lee, Bolles: The Microtomists Vade-Mecum, ed. 10, Philadelphia, The Blakiston Company, 1937.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3. Rinse twice in distilled water quickly in the dark and transfer to a freshly prepared solution of

Hydroquinone	1 gm.
Sodium sulfite	0.15 gm.
Distilled water	85 cc.
Neutral formol (40% formaldehyde)	15 cc.

Leave in this solution for 12-24 hours, and then wash three times with distilled water for 10 minutes in each wash.

4. Employ the same dehydrating and embedding techniques used previously. Do not add any iodine to any of the alcohols.

Staining.—Staining is done as follows:

1. Hydrate.

2. Treat with gold chloride, 1 gm. in 500 cc. of water, for ½ to 1 hour. Check under the microscope until toning is completed (Golgi apparatus is black).

3. Rinse in distilled water briefly.

4. Treat with sodium hyposulfite 5% for 5 to 15 minutes.

5. Wash in tap water for 15 minutes.

6. Counterstain with Masson trichrome or Gomori's hematoxylin.

7. Dehydrate, clear, and mount as before.

The Mallory-Heidenhain azocarmine method can also be used for counterstaining. Counterstaining with Gomori's hematoxylin can be performed without any modification. Counterstaining with the trichrome method can be done without modification if started at Step 13. The results, however, are not as warm as when the following method is used. After Step 5 of the Aoyama procedure do Step 6 to 9 of the regular trichrome procedure and then differentiate the nuclei as follows:

1. Rinse with distilled water until no more stain comes away.

2. Differentiate the nuclei with 1% iron-alum. The slides must be shaken continuously in order to obtain an even differentiation. Control the reaction under the microscope with the use of distilled water.

3. Rinse with distilled water and wash in running water for 15-20 minutes.

4. Dehydrate, clear, and mount as before.

Results.—The Golgi apparatus is black. The cells are stained in the usual manner of the counterstain, but because of formaldehyde fixation the cytoplasmic details are not as well

EXPLANATION OF FIGURE 2

A, photomicrograph of a mitochondrial preparation of a pancreatic islet from a rabbit at birth. The mitochondria appear as fine black filaments. The granules of the alpha cells also stain black, and it is not possible to distinguish their mitochondria in these preparations. Such a black alpha cell may be seen at the apex of the islet.

Coarse spherical black zymogen granules and filamentous mitochondria are seen in the acinar tissue.

The section is 2.5 μ in thickness. × 1,200.

B, photomicrograph of pancreatic islet from a rabbit 1 year old, stained by the Aoyama method for the Golgi apparatus and counterstained by the trichrome technique. Both alpha cells (red-brown) and Golgi apparatus (black) appear black in the photograph; beta cells (pink) and gray and granular, while delta cells are pale and agranular. In the center of the picture is a crescent of black alpha cells. The crescent is formed by a tangential section through the tips of several alpha cells. No nuclei have been sectioned. Lying in the hollow of the crescent close to the tip of its lower arm is a dark granular alpha cell, and close to its nucleus is a compact homogeneous black mass that represents the Golgi apparatus. Immediately above and to the right of the crescent of alpha cells lies a large pale delta cell in which can be seen a few small black filaments that constitute its Golgi network. The irregular, branched Golgi apparatus of the beta cells can be seen in the many cells present. Its heaviest portion lies close to the nucleus and corresponds to the negative Golgi image seen in ordinary preparations.

The section is 2.5 μ in thickness. × 1,200.

defined as if Zenker-formol fixed tissues had been employed. Nevertheless, an exact differentiation of the alpha, beta, and delta cells is possible, and their Golgi apparatus are demonstrated at the same time.

This procedure has proved to be of great help in the study of the embryonal islet cells. It is perfectly applicable when pathologic tissues are to be studied.

Procedure for the Demonstration of the Argentaffinity of the Islet Cells in Paraffin Section

1. Fix in Bouin's trichloroacetic acid mixture or in formalin 10%.

2. Dehydrate, clear, embed, and cut in the usual manner.

3. Stain with (*a*) Fontana's stain (for argentaffin cells, Masson's modification[3a]) or (*b*) Foot-Laidlow reticulin stain (Masson's modification[3b]) or (*c*) Roger's silver method as modified by Van Campenhout[11] for nerve impregnation.

4. (*a*) Counterstain with the trichrome technique, differentiating with 1% iron-alum. (*b*) If nuclear detail is not important, the counterstain may be started at Step 13 of the Masson trichrome stain.

Results.—Argentaffin cells are black, while the remainder of the tissue has the usual differential characteristics of the trichrome method.

COMMENT

In the course of a study of the staining methods best suited to the cytological investigation of the pancreas it was found that Masson's trichrome stain could be developed into an excellent general survey stain that clearly differentiates alpha, beta, and delta cells. It was also found that both the trichrome and the Gomori method could be used to counterstain differentially Golgi preparations done by the Aoyama technique and to counterstain argentaffin preparations done by Roger's, Foot-Laidlow, or Fontana's technique. These various methods, together with that of Schridde for mitochondria, proved adequate for the study of the embryonic pancreas as well as for that of the normal and pathological adult pancreas.

The results of the method detailed above are most typical of the rabbit's pancreas. The human pancreas can be treated in exactly the same manner, with excellent results. I have found it necessary to alter the time schedules of some of the procedures when applying the methods to the pancreases of birds, fish, turtles, and other vertebrates, but it has seldom been necessary to change the concentrations of the reagent and the staining solutions. It may be noted that embryos and lower forms of vertebrate life fix more quickly than adult tissues or tissues from higher forms.

It has been observed that the trichrome method will stain certain cells or parts of cells somewhat differently, even in the same islet. The tinctorial variations have been studied in combination and correlation with the various other techniques described above, and it has been found that most of the color variations are specifically associated with easily demonstrable changes in various cellular organelles. This fact has made the trichrome method extremely useful, since it is possible to employ it as a survey stain that detects simultaneously all of the cytoplasmic elements that may be demonstrated by other methods.

Satisfactory results can be obtained only by following the techniques faithfully in every detail. It has been found that carelessness at one stage of the procedure cannot be compensated for by great care at some subsequent stage. A series of minor faults accumulate to constitute a major defect. If the investigator will acquaint himself with the minutiae of the techniques he will have at his disposal methods of high specificity, great elasticity, and easy reproducibility.

SUMMARY

A systematic investigation of the various methods available for the cytological study of the islets of Langerhans of the pancreas resulted in the modification and combination of a small group which proved to be suitable for the detailed study of the normal, pathological, embryonic, or adult pancreas.

It was found that Masson's trichrome stain could be developed into an excellent survey stain that not only clearly differentiated alpha, beta, and delta cells but also detected simultaneously all of the cytoplasmic elements that may be demonstrated more clearly by other methods. Both the Masson trichrome and the Gomori method can be applied successfully to counterstain differentially preparations in which the Golgi apparatus has been shown by the Aoyama technique or to counterstain argentaffin preparations done by Roger's, Foot-Laidlaw and Fontana's techniques. The Schridde method was used to demonstrate mitochondria.

These various methods used alone and in combinations allow unusually complete and exact observations to be made on the pancreas.

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News and Notes

Questions on Exfoliative Cytology.—The American Board of Pathology will include questions on exfoliative cytology in both the written and the practical examination, starting in the spring of 1953.

American Academy of Forensic Sciences.—The fourth annual meeting will be held March 6, 7, and 8, 1952, at the Biltmore Hotel, Atlanta. All those interested in presenting papers are urged to submit their titles immediately to Dr. A. W. Freireich, Program Chairman, 180 Hempstead Avenue, Malverne, N. Y.

Books Received

A Textbook of Pathology. By Robert Allan Moore, Edward Mallinckrodt professor of pathology, Washington University School of Medicine, St. Louis. Second edition. Pp. 1048, with 501 illustrations. Price $12.50. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1951.

The vast amount of new and changing information which has accrued since the publication of this textbook in 1945 makes the second edition a welcome supplement to current pathologic literature. The general plan and scope of the book are the same as the first edition, but there has been extensive reorganization of topics and inclusion of new material. The introduction of the double-column format is functionally advantageous to the reader, and has permitted a reduction, relative to the original volume, of 290 pages. The result is a text of pleasing and manageable proportion.

New chapters have been added on disturbances in metabolism of enzymes, on diseases peculiar to the aged, and on general considerations of infectious diseases. Newer concepts concerning the diffuse collagen diseases and the demyelinating encephalitides have been awarded separate chapter headings. Additional new material has been included on virus diseases, diseases of the blood, and bone and joint lesions. The scattered discussions of renal diseases found in the previous edition have been assembled to improve correlation, while the sections on virus diseases, the digestive tract, and the skin have been subdivided to provide succinct study units. However, thromboangiitis obliterans and Raynaud's disease remain dangling in the chapter on arteriosclerosis.

The total number of illustrations has been reduced from 513 to 501, with the deletion of figures of secondary interest. The black and white photographs are of excellent quality with only a few exceptions (Figs. 84, 154, and 210 have been reproduced rather poorly and Figs. 46 and 81 are of questionable value), and the many colored illustrations are fresh and brilliant.

The lists of English-language references following each chapter have been frequently supplemented by pertinent new articles, and their value thereby enhanced.

Minor deficiencies are encountered, which do not greatly detract from the general excellence of the work. Closer attention by proofreaders and typesetters will eliminate the relatively frequent errors in spelling, the awkward hyphenations, and occasional grammatical inconsistencies. The legends under Figures 406 and 407 have become reversed. It is felt that the important chapter on congenital heart disease could be reorganized and modernized to great advantage. Neither embryologic nor phylogenetic emphasis is used to guide the reader on an integrated survey of the several diverse types of cardiac defects chosen for discussion. From the pedagogic standpoint the practice of introducing new diseases with a description of the pathologic anatomy, followed by the etiology and the pathogenesis, may be questioned. Statements of fact, bolstered only by reference to an author's name, are frequently made with an implication of finality that is not always justified by the controversial nature of the subject. These defects are attributable, in part, to the difficult task of furnishing a semblance of completeness in a student text.

This book has been written primarily for the student, but its use will richly repay the medical practitioner as well. It is remarkably complete and up-to-date. The many refreshing innovations in its approach to the subject matter, with emphasis on the dynamic and the clinical aspects of disease, will continue to make this text a popular and stimulating one for the student of pathology.

Approved Laboratory Technic. John A. Kolmer, M.D., D.P.H., Sc.D., professor of medicine and director of the Institute of Public Health and Preventive Medicine, Temple Uni-

versity; formerly professor of pathology and bacteriology, University of Pennsylvania Graduate School of Medicine; Earle H. Spaulding, Ph.D., professor of bacteriology, Temple University School of Medicine; and Howard W. Robinson, Ph.D., professor of physiological chemistry, Temple University School of Medicine. Ed. 5. Pp. 1,180, with 403 illustrations and 28 color plates and 78 tables. Price $12. Appleton-Century-Crofts Company, Inc., 35 W. 32d St., New York 1; 31-32 Bedford St., Strand, London, W.C. 2, 1951.

With the death of Dr. Boerner, the fifth edition of this standard work on laboratory technique has now a change of authorship. There have been numerous textual changes. The new edition shows extensive inclusion of new techniques such as cytological diagnosis, new pregnancy tests, liver function tests, newer methods of utilization of recently developed antibiotics and their assay, to name but a few. Almost every chapter has been revised, and the entire gamut of laboratory procedures is covered. This reviewer has always found the previous editions particularly useful in the fields of bacteriology and serology, and here the enlargements have been especially welcome.

A question may be raised, however, concerning the purpose underlying any book on technique. Is it a textbook for a beginner's course in medical technology? Is it a reference book for the experienced technician? Is it a manual for the clinical pathologist who supervises the laboratory? Or is it trying to serve all purposes? It may be questioned whether this can any longer be done within the confines of a single volume. There seems no reason for including such advanced chemical techniques as the determination of serum copper, iron, and magnesium in the same volume with pictures of a microscope lamp. As a book grows, there must be a more deliberate awareness of the audience for which it is written. If the volume is intended to be a text for student technicians, the emphasis and detail would need to be adjusted and a great deal could be omitted. Even for experienced technicians there is much material included that is scarcely of practical importance. Or, if the volume is intended as an encyclopedic reference, some of the methods are now given in too scanty detail to be practically useful.

As knowledge continues to expand, future editions of this excellent book might well take the form of more careful address to a particular audience. More probably it should take the form of two volumes, an elementary and an advanced section, with different emphasis and amount of detail in each.

Surgical Practice of the Lahey Clinic. By Members of the Staff of Lahey Clinic, Boston. Pp. 1014, with 784 illustrations on 509 figures. Price $15. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1951.

The Specialties in General Practice. Edited by Russell L. Cecil, M.D., professor of clinical medicine, emeritus, Cornell University Medical College, New York City. With articles by 14 contributors. Pp. 818, with 470 illustrations. Price $14.50. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1951.

Physical Medicine and Rehabilitation for the Clinician. Edited by Frank H. Krusen, M.D. With articles by 24 contributors. Pp. 371, with 96 illustrations and 12 tables. Price $6.50. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1951.

Die generalisierten Tuberkulosen. By Dr. Med. F. Schmid, Universitäts-Kinderklinik, Heidelberg, Germany. Pp. 230, with 60 illustrations. Price 24 German marks. Georg Thieme, Diemershaldenstrasse 47, (14a) Stuttgart-O; agents for U. S. A.: Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1951.

Late Prognosis in Meningococcal Meningitis. By Elli Trolle. Pp. 310. Paper bound. Acta psychiat. et neurol., Supp. 66, Copenhagen, Danish Science Press, Ltd., 1951.

Thyroid Function and Its Possible Role in Vascular Degeneration. William B. Kountz, M.D., assistant professor of clinical medicine, Washington University School of

Medicine; director of clinical services, Division of Gerontology, Washington University School of Medicine, and the St. Louis City Infirmary Hospital; consulting physician, Barnes Hospital and Lutheran Hospital, St. Louis. Pp. 62, with 10 illustrations. Price $2.25. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1951.

COMPARATIVE PHYSIOLOGY OF THE THYROID AND PARATHYROID GLANDS. By Walter Fleischmann, M.D., Ph.D., Veterans Administration Hospital, Fort Howard, Md., instructor in pediatrics, The Johns Hopkins University School of Medicine, Baltimore. Pp. 78. Price $2.25. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1951.

A COLOR ATLAS OF MORPHOLOGIC HEMATOLOGY WITH A GUIDE TO CLINICAL INTERPRETATION. By Geneva A. Daland, B.S., chief laboratory assistant in hematology, Thorndike Memorial Laboratory; research laboratory technician, Boston City Hospital. Edited by Thomas Hale Ham, M.D., assistant professor of medicine, Harvard Medical School; associate director, Thorndike Memorial Laboratory; junior visiting physician, Boston City Hospital. Illustrations by Etta Piotti. Pp. 74, with 14 plates and 12 tables. Price $5. Harvard University Press, Cambridge 38, Mass., 1951.

This atlas, which is written by a qualified technician in hematology, furnishes a guide for reference in the study of films of the peripheral blood stained with Wright's stain. The description of the blood patterns encountered in a variety of clinical conditions is evaluated from a diagnostic point of view. The maturation of the several series of blood cells is briefly described and shown in diagrams and color plates. Techniques and laboratory procedures are limited to those that aid in the diagnosis of the blood disorders with characteristic blood patterns. The morphologic aspects and the clinical interpretations of the blood films from patients with common types of anemia, leukemias, infectious mononucleosis, and other hematologic conditions are emphasized and illustrated with a series of color plates. The views expressed by the author on the subject of erythropoiesis are not in agreement with those generally held. She overlooks the clinical and therapeutic implications of separating normoblastic and megaloblastic types of erythropoiesis. This atlas should be useful to those for whom it is intended—the technologist, the medical student, and the physician in general practice.

ATLAS OF TUMOR PATHOLOGY. Section IX, Fascicle 34: Tumors of the Breast. By Fred W. Stewart, M.D., pathologist to Memorial Hospital for the Treatment of Cancer and Allied Diseases; professor of pathology, Cornell University Medical School; attending pathologist, New York Hospital, New York. Pp. 114, with 68 illustrations. Price $1.10. Published by the Armed Forces Institute of Pathology under the Auspices of the Subcommittee on Oncology of the Committee on Pathology of the National Research Council, Washington, D. C., 1950.

Dr. Stewart's enormous experience of breast tumors is reflected in this monograph. In a short introduction he gives the classification employed at the Memorial Hospital, and then, in the course of the atlas, he discusses and illustrates the different subdivisions. The division is admittedly imperfect, for overlap is not avoided, but Stewart truly says, "Cancers of the breast simply do not adjust their growth pattern to the requirements of rigid coding." The general scheme includes, first, Paget's disease, then carcinomas of duct origin, then those of the lobule. Successive subdivisions are "relatively rare carcinoma," followed by "malignant cystosarcoma phyllodes." Then are taken up various types of sarcoma, and finally "combined forms of the above." The discussion is generally succinct, and the illustrations of uniformly high quality. There is also excellent discussion of lesions simulating carcinoma, such as fibrosing adenosis.

Stewart holds that "appropriate anatomical histological classification obviates the need for grading" of tumors. The pragmatic importance of classification is therefore stressed. Quite certainly his own classification will not meet with universal approval, but through such differences of opinion is progress made. The monograph, although slightly tinged with dogmatism, should be on the bookshelf of every practicing pathologist.

PSYCHOSOMATIC GYNECOLOGY: INCLUDING PROBLEMS OF OBSTETRICAL CARE. By William S. Kroger, M.D., assistant clinical professor of obstetrics and gynecology, Chicago Medical School; attending obstetrician and gynecologist, Edgewater Hospital, Chicago; and S. Charles

Freed, M.D., adjunct in medicine, Mount Zion Hospital, San Francisco. Pp. 503. Price $8. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1951.

STUDIES IN THE HISTOLOGY OF EARLY LESIONS IN LEPROSY. By V. R. Khanolkar, M.D., director of laboratories, Tata Memorial Hospital, Bombay, India. Pp. 32, with 30 illustrations. Price, 2 rupees, 8 annas (2-8-0). Indian Council of Medical Research, Special Report Series, No. 19, New Delhi, 1951.

Khanolkar has studied the development of leprosy from the stage of contact in individuals with a positive lepromin test but with no clinical evidence of leprosy up to the stages of classic lepromatous or tuberculoid leprosy. In contacts without lesions and with a positive lepromin test, he finds a few scattered histiocytes in the skin either with an engulfed acid-fast bacillus or with granular opaque acid-fast particles in their cytoplasm. With the development of the earliest macular lesions, bacilli and lesions are demonstrated to be in intimate relationship with terminal nerve radicles in the skin, and the differences of reaction between lesions which will become lepromatous and lesions which will become tuberculoid are described. The further development of these lesions to become those of the early lepromatous or tuberculoid stages is traced, and again the intimate involvement of nerve fibers is carefully documented. This monograph is a valuable addition to our knowledge of the genesis of leprous lesions and deserves careful reading by all those interested in this subject.

PEPTIC ULCER: CLINICAL ASPECTS—DIAGNOSIS—MANAGEMENT. Edited by David J. Sandweiss, M.D., associate attending physician, Division of Internal Medicine, Harper Hospital, Detroit. Pp. 790, with 164 illustrations. Price $15. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1951.

TEXTBOOK OF REFRACTION. By Edwin Forbes Tait, M.D., Ph.D., associate professor of ophthalmology, Temple University School of Medicine; attending surgeon (ophthalmology), Temple University and Montgomery Hospitals; fellow, Philadelphia College of Physicians and American Academy of Ophthalmology and Otolaryngology; member of the Pan-American Association of Ophthalmology and the Association for Research in Ophthalmology. Pp. 418, with 93 illustrations. Price $8. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1951.

ATLAS OF TUMOR PATHOLOGY. Section V, Fascicle 18: Tumors of the Mediastinum. By Hans George Schlumberger, M.D., professor of pathology, Ohio State University College of Medicine. Pp. 88, with 75 illustrations. Price 75 cents. Published by the Armed Forces Institute of Pathology under the Auspices of the Subcommittee on Oncology of the Committee on Pathology of the National Research Council, Washington, D. C., 1951.

Tumors of the mediastinum is a subject very difficult to treat in any unified fashion, because of diverse histogenesis of the elements giving rise to the tumors. This fascicle in dealing with the mediastinum takes up tumors of nervous tissue; those of mesenchymal derivations; those of displaced tissues (thyroid, parathyroid, teratoma); tumors of hematopoietic tissues; mediastinal cysts, and, finally, miscellaneous lesions. Schlumberger has performed a splendid task in presenting a difficult subject. It might be suggested that the illustrations include too many x-rays and too many photographs of gross specimens, whereas a somewhat wider selection of microscopic examples might have been more welcome. There are good bibliographies.

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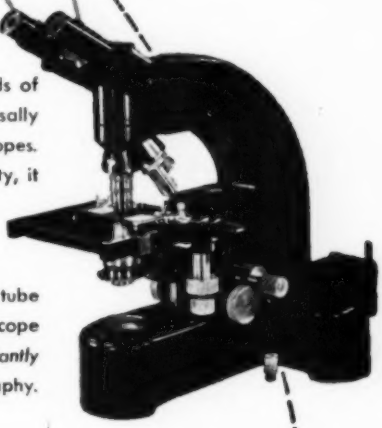

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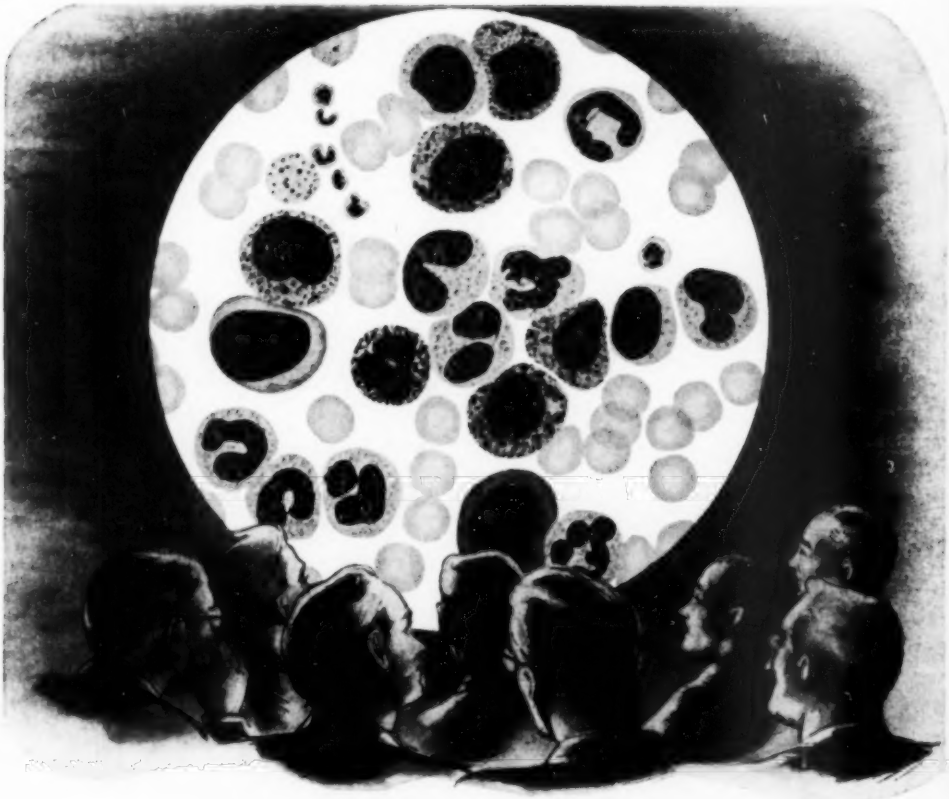

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